SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF PALM CATANIONIC SURFACTANTS

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ABSTRACT

Surfactant is well known for its versatility in many forms of applications. Today, new types of surfactants continue to emerge and evolve to cater to the changing needs of industries. One of these interesting and promising new surfactants is catanionic surfactant. Its unique aggregation behaviour and superior surface activities have sparked interests to many researchers in the area of colloidal chemistry.

In our study, C_mMES.C_nTAB catanionic surfactants were formed by mixing equimolar ratio of cationic surfactant alkyltrimethylammonium bromide (C_nTAB) and anionic surfactant sodium methyl ester α-sulfonate (C_mMES). Precipitation method coupled with cold filtration system was employed to obtain the white precipitate of C_mMES.C_nTAB catanionic surfactants. All samples were characterized by fourier transform infrared and NMR spectroscopies. Spontaneous formation of vesicles of C_mMES.C_nTAB catanionic surfactants was also observed under optical polarizing microscope.

The surface properties of binary mixtures of anionic sodium methyl ester α-sulfo alkylate (C_mMES) and cationic alkyltrimethylammonium bromide (C_nTAB) of different carbon chain length were studied using tensiometer via du Nuoy ring method. The critical micelle concentration (CMC) obtained from the plots of surface tension (γ) versus concentration showed that mixed surfactants have CMC values of about 10 times lower than their single components. The large negative values for both interaction parameters suggest the existence of strong synergism between the oppositely charged surfactant molecules. The average interaction parameter values are approximately -14. Effect of hydrocarbon chain length of either surfactant was also compared and the

results showed that the effect of cationic surfactant chain length dominated that of the anionic surfactants. It was also discovered that certain mixed surfactant combinations behave differently from the expected trend.

Aggregation behaviour of C_mMES.C_nTAB catanionic surfactants at the air/water interface were explained by surface tension measurement technique. Surface activities of catanionic surfactants were extremely high with critical micelle concentration (CMC) values from as low as 0.01 mM. This is further confirmed by the surface excess concentration, Γ_{max} and minimum area, A_{min} values of the catanionic surfactants. Besides that, catanionic surfactants with lower alkyl chain length were found to have greater ability in decreasing the air/water interfacial tension.

Anionic/cationic mixtures and catanionic surfactants were also bioassayed on both Gram-positive (*Staphylococcus aureus*, SA) and Gram-negative (*Escherichia coli*, EC and *Pseudomonas aeruginosa*, PA) using broth dilution method. Both anionic/cationic mixed surfactants and catanionic surfactant solutions showed excellent in-vitro antibacterial activity against SA ranging from 1.25 to 125 μM and had variable activity against EC with minimum inhibitory concentration (MIC) of 10 to 1250 μM but were less active against PA. Inhibitive power of anionic/cationic mixtures was on par with single C_nTAB surfactants; with certain mixtures even have lower minimum inhibition concentration (MIC) than single C_nTAB surfactants. Besides that, anionic/cationic mixed surfactants at equimolar mixing ratio showed exceptional inhibitive power against SA and EC. Although catanionic surfactants have higher surface activities, but they were less potent towards all three microbes as compared to its anionic/cationic mixtures.

ABSTRAK

Surfaktan terkenal dengan kepelbagaian bentuk aplikasinya. Jenis surfaktan yang baru terus ditemui dan senantiasa mengalami evolusi untuk menampung keperluan industri semasa. Salah satu jenis surfaktan baru yang manarik dan berpotensi adalah surfaktan katanionik. Keunikan sifat agregasinya dan aktiviti permukaan yang baik telah melahirkan minat di kalangan penyelidik dalam bidang kimia koloid.

Dalam kajian ini, surfaktan katanionik C_mMES.C_nTAB telah dihasilkan daripada pencampuran surfaktan kationik, alkiltrimetilammonium bromida (C_nTAB) dan surfaktan anionik, sodium metil ester α-sulfonat (C_mMES) dalam nisbah 1:1. Kaedah pemendakan beserta sistem penapisan sejuk telah digunakan untuk memperolehi surfaktan katanionik C_mMES.C_nTAB dalam bentuk mendakan putih. Semua sampel dicirikan dengan kaedah spektroskopi infra merah dan NMR. Pembentukan 'vesicles' surfaktan katanionik C_mMES.C_nTAB secara spontan juga diperhatikan di bawah mikroskop optik polarisasi.

Ciri-ciri permukaan campuran binari anionik sodium metil ester α-sulfo alkilat (C_mMES) dan kationik alkiltrimetilammonium bromid (C_nTAB) yang terdiri daripada pelbagai rantai karbon telah dikaji dengan menggunakan tensiometer melalui kaedah bulatan du Nuoy. Kepekatan kritikal misel (CMC) yang diperolehi daripada plot ketegangan permukaan (γ) berbanding kepekatan surfaktan menunjukkan bahawa surfaktan campuran mempunyai nilai CMC yang 10 kali lebih rendah daripada surfaktan induknya. Nilai negatif yang besar daripada kedua-dua parameter interaksi mencadangkan kewujudan tenaga sinergistik yang kuat antara molekul-molekul surfaktan yang bertentangan caj. Nilai purata parameter interaksi adalah kira-kira -14.

Kesan kepelbagaian rantai karbon daripada kedua-dua surfaktan juga telah dibandingkan dan keputusannya menunjukkan bahawa kesan kepanjangan rantai karbon surfaktan kationik adalah lebih dominan daripada surfaktan anionik. Selain itu, sesetengah surfaktan campuran juga didapati mempunyai ciri-ciri permukaan yang berlainan daripada jangkaan.

Ciri-ciri pengumpulan sukfaktan katanionik C_mMES.C_nTAB di permukaan air telah diterangkan menggunakan teknik pengukuran ketegangan permukaan. Aktiviti-aktiviti permukaan surfaktan katanionik adalah sangat tinggi di mana nilai CMC adalah serendah 0.01 mM. Ia juga dibuktikan dengan nilai kepekatan berlebihan permukaan, Γ_{max} dan keluasan minimum, A_{min}. Tambahan pula, surfaktan katanionik dengan rantai karbon yang lebih pendek mempunyai kebolehan yang lebih besar dalam pengurangan ketegangan permukaan air.

Ciri-ciri biologi surfaktan campuran anionik/kationik dan katanionik juga dikaji dengan menggunakan kedua-dua bakteria Gram-positif (*Staphylococcus aureus,* SA) dan Gram-negatif (*Escherichia coli*, EC and *Pseudomonas aeruginosa*, PA) melalui kaedah pencairan larutan. Kedua-dua surfaktan campuran anionik/kationik dan katanionik menunjukkan aktiviti anti-bakteria in-vitro yang baik terhadap SA di mana nilai perencatan minimum (MIC) adalah sekitar 1.25 - 125 μM, manakala aktivitinya terhadap EC adalah sekitar 10 – 1250 μM. Aktiviti anti-bakteria kedua-dua surfaktan adalah rendah terhadap PA. Kuasa perencatan campuran anionik/kationik adalah setanding dengan surfaktan individu, C_nTAB. Terdapat juga beberapa campuran yang mempunyai MIC yang lebih rendah daripada C_nTAB. Selain itu, campuran anionik/kationik dalam nisbah sama molar mempamerkan kuasa perencatan yang amat tinggi terhadap SA dan EC. Walaupun surfaktan katanionik mempunyai aktiviti

permukaan yang lebih tinggi berbanding campuran anionik/kationik, tetapi kuasa perencatan surfaktan katanionik terhadap ketiga-tiga bakteria adalah lebih rendah.

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ABBREVIATIONS

℃	degree Celsius
%	per cent
ΔG°_{ex}	excess Gibbs free energy of mixing
β	interaction parameter
β^{mic}	interaction parameter in micelle
β^{mon}	interaction parameter at surface monolayer
Γ_{max}	surface excess concentration
μm	micrometer
γ	surface tension
^{13}C NMR	carbon-13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
$2C_{18}C_{12}$	dioctadecyldimethylammonium bromide
A_{min}	minimum area per molecule
ATPS	aqueous two phase system
BAC	benzalkonium chloride
BSA	benzene sulfonic acid
BzSo	benzenesulfonate
C	concentration
cfu	colony forming unit
$C_{10}CX$	decylcarboxylate
$CDCl_3$	deuterated chloroform
CMC	critical micelle concentration
CMC*	ideal critical micelle concentration
C_mMES	sodium alkyl methyl ester sulfonate

C_nNB	alkyltributylammonium bromide
C_nNE	alkyltriethylammonium bromide
C_nNM /C_nTAB	alkyltrimethylammonium bromide
C_nSO_3	alkyl sulfonate
C_nSO_4	alkyl sulfate
CPC	cetylpyridinium chloride
CTABr/CTAB	cetyl trimethylammonium bromide
CTAT	cetyl trimethylammonium tosylate
DDAB	didodecyldimethylammonium bromide
DSC	differential scanning calorimeter
EC	Escherichia *coli*
FTIR	Fourier transform infrared
FT-NMR	Fourier transform- nuclear magnetic resonance
g	gram
$g_{1/}$ g_2	activity coefficient
IC_{90}	concentration where there is decrease of 90% in bacteria colonies
K	Kelvin
KBr	potassium bromide
LAS	alkylbenzene sulfonate
LB	Langmuir-Blodgett
MHz	mega hertz
mg	miligram
MIC	minimum inhibition concentration
min	minute
mm	milimetre
mM	milimole per decimetre^3

m N/m	miliNewton per metre
mL	mililitre
MPOB	Malaysian Palm Oil Board
N_A	Avagadro's number
NaBr	sodium bromide
nm	nanometre
PA	Pseudomonas . *aeruginosa*
PBSo	hexadecylpyridinium 4-octylbenzenesulfonate
PS	hexadecylpyridinium octylsulfate
PSo	hexadecyl pyridinium octylsulfonate
QAC	quaternary ammonium compound
R	gas constant
rpm	rotation per minute
SA	Staphylococcus *aureus*
SDBS	sodium dodecylbenzenesulphonate
SDS	sodium dodecyl sulfate
SMS	sodium methylate sulfate
TABSo	hexadecyltrimethylammonium 4-octylbenzenesulfonate
TAS	hexadecyltrimethylammonium octylsulfate
TASo	hexadecyltrimethylammonium octylsulfonate
TMS	tetramethysilane
TSA	Trypticase soy agar
wt%	weight per cent
X_{mic}	mole fraction of surfactant 1 in the mixed micelle
X_{mon}	mole fraction of surfactant 1 in the mixed monolayer
XRD	x-ray diffraction

CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction to Surfactants

Surfactant is a shortened term of the phrase "surface-active agent". Surfactants tend to accumulate at surfaces or interfaces and affect the surface characteristic of the system to a marked degree. This amphiphilic behaviour is contributed by the presence of two moieties in the molecular structure of a surfactant, namely hydrophilic head group and hydrophobic tail (Figure 1.1). The driving force comes from the hydrophobic section where it will move away from the solvent and drive the surfactant into self-association (Clint, 1992).

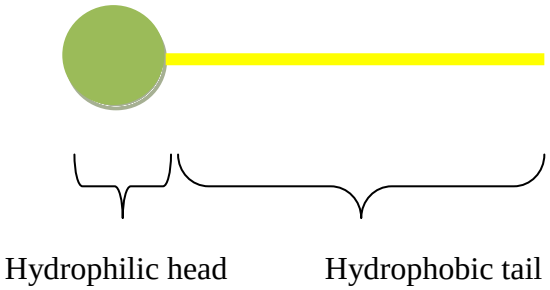


Figure 1.1 : Schematic diagram of a surfactant molecule.

Generally, surfactants are employed to increase the contact of two materials which can be solid/liquid, liquid/liquid or liquid/gas. Surfactants and surface activity are controlling features in many important systems, such as emulsification, detergency, foaming, wetting, lubrication, water repellence, waterproofing, spreading and dispersion, and colloid stability in many industrial processes and formulated products. Today, worldwide consumption of surfactants amounts to several million tonnes (Tadros, 2005).

In most cases, surfactants are synthetic rather than naturally occurring. Petroleum derived feedstocks (i.e. alcohols, alkylbenzenes, alkylphenols) and natural raw materials (vegetable and animal-derived oils and fats) are commonly used to produce synthetic surfactants. The first production of surfactant can be dated back to the Roman times when soap was first made (Clint, 1992).

1.2 Types of Surfactants

Surfactants are classified according to the composition of their hydrophilic head group. A nonionic surfactant has no charge group on its head. Ionic surfactants that have positive charge are called cationic surfactants while negatively charged surfactants are called anionic surfactants. Surfactants that have both positive and negative charge on its head are termed zwitterionic surfactants. Different types of surfactants have different characteristics and are useful in their own way. For example, alkyl trimethylammonium bromide, a type of cationic surfactant has good detergency properties, softening effect and also can be used as a disinfectant. The sodium salt of linear alkyl benzenesulfonates, an anionic surfactant, is widely used in industrial and high-foaming household detergents and cosmetics. Meanwhile, nonionic surfactants such as polyoxyethylene glycol esters are commonly used as emulsification agents in cosmetic products. Zwitterionic surfactants, β-N-alkylaminopropionic acids, have a broad spectrum of applications ranging from bactericides, corrosion inhibitors, dispersion agents, and cosmetics to alkaline cleaner (Rosen, 1989).

On the other hand, differences in the hydrophobic region also affect the characteristic of a particular surfactant. For instance, longer hydrocarbon chain length increases the interfacial activities and causes closer packing of the surfactant monomers. Whereas, the introduction of branching or unsaturation in the hydrophobic region increases

solubility and facilitates looser packing of the monomers. Addition of aromatic nucleus may increase its tendency to adsorb on polar surfaces. Presence of polyoxypropylene chain was also known to increase the adsorption of the surfactant to polar surfaces via the hydrophobic part. On top of these, addition of perfluoroalkyl or polysiloxane group helps to lower the surface tension of water. However, such differences are less dominant as compared to the hydrophilic group (Rosen, 1989).

The ability to tune the characteristic of surfactants according to the desired applications is a major area to researchers to keep on investigating in this field. In terms of surface properties, scientists are keen to produce a surfactant system that occupies the smallest area at the interface, as this can lower the tension between the two media (Rosen, 1989).

In other works, researchers also tried to modify the aggregations behaviour to better suit the application such as vesicles for drug delivery (Guo & Szoka, 2002). Besides that, charges on the surfactant head can also be modified to better suit the target surfaces or simply enhance the performance of the surfactants.

1.3 Mixed Surfactants

Apart from the four common types of surfactants (non-ionic, anionic, cationic and zwitterionic), various type of new surfactants have emerged in recent years. For instance, biosurfactants, catanionic mixtures and gemini surfactants. Besides this, mixed surfactants (anionic- anionic, cationic-cationic, cationic-nonionic, anionic-nonionic and nonionic-nonionic) were also prepared to study of their properties. Many researchers have found that the mixed systems showed better surface activities as compared to single surfactants (Murphy and Taggart, 2002; Frese *et al*., 2003).

As compared to single surfactant systems, the phase behaviour of mixed surfactants is significantly more complex. This is due to the mixing of different surfactant monomers are rarely ideal. Interactions are that present between the different surfactant monomers could be antagonistic or synergistic. Such interactions may lead to the formation of certain unique microstructures which are not found in any single surfactant systems. Discovery of these new microstructures have brought surfactants to a whole new level of versatility.

Most surfactants used in practical applications actually consist of mixtures of surfactants, since these often perform better than the individual components. Rodlike micellar solutions have found use as drag-reduction agents in pipeline flow (Israelachivili, 1992) and stable vesicle phases are of potential use as microreactors and agents for controlled drug release (Ostro, 1987). Dilute liquid crystals can be utilised in the formulation of liquid detergents (Khan & Marques,1997). The precipitation of mixed surfactants is useful in the quantitative analysis of surfactants. In order to develop these and other applications, it is essential to understand how surfactants interact with each other and how these interactions influence the performance and phase equilibria of the surfactant systems.

Numerous modelling methods had been employed to study on the micellization behaviour of mixed surfactant systems. For example, pseudophase separation approach, ideal mixing models, nonideal mixing models, mass action models, molecular models and so on. Generally, the selection of approach is based on the complexity of the mixed systems and level of detail desired. There is no one model that can represent all the mixed systems due to the different nature of the mixed systems (Holland and Rubingh, 1992).

1.3.1 Mixed Surfactants of the Same Charges

When two surfactants of like charges (anionic/anionic, cationic/cationic and non-ionic/non-ionic surfactant) are mixed together, the physical properties of the mixtures are intermediate between the pure components, or show only a very little deviation. These systems are normally classified as ideal systems with near zero values for interaction parameter, β (Murphy & Taggard, 2002; Vora *et al*., 1999). However, if the surfactants have a more dissimilar structure or different counterions, the physical properties of the mixtures will show higher deviation from either pure component. As compared to other types of mixtures, these mixed systems of the same charges exhibited smallest deviation from ideality and hence showed little improvement on surface activities (Murphy & Taggard, 2002; Holland, 1992).

1.3.2 Ionic/Non-ionic Mixtures

Binary mixtures of anionic/non-ionic, cationic/non-ionic and zwitterionic/non-ionic are classified under this category. As these mixed systems consist of an ionic and a non-ionic head group, electrostatic repulsions between the surfactant molecules are reduced. Such phenomena contribute to a closer packing of the surfactant molecules and this leads to greater surface activities (Rosen, 1989). Much larger deviation from ideality was observed for these mixed systems as compared to mixtures of same charges (Vora *et al*., 1999). Several investigations also shown that values of interaction parameter, β are slightly negative (Haque *et al*., 1996; Jańczuk *et al*., 1995; Holland, 1992). Plenty of industrial applications favour these mixed systems due to its higher surface activities and lower tendency of such mixtures to form precipitates (Sharma *et al*., 2004; Holland, 1992).

1.3.3 Anionic/Cationic Mixtures

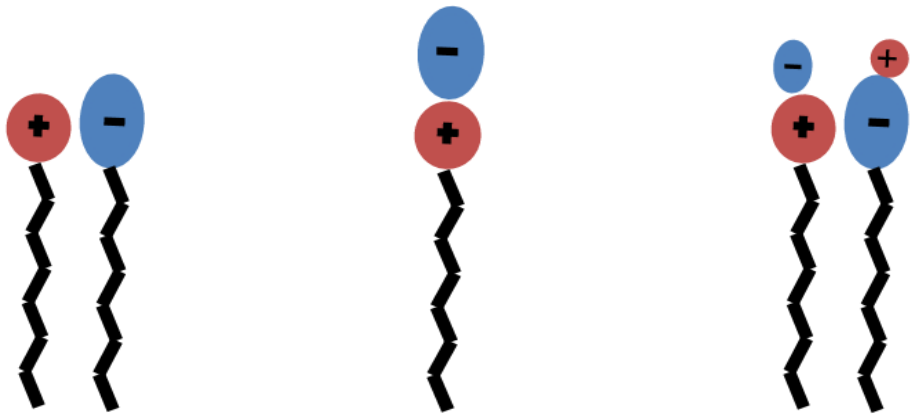
Mixtures of surfactants have been studied extensively for their phase equilibria, physico-chemical properties and applications over the years but there are not many studies of systems where both a cationic and an anionic surfactant are mixed with water. This is due to the low solubility of anionic/cationic complexes (1:1 molar ratio) in aqueous solution which could render the system useless. However, interest on this anionic/cationic mixed system escalated quickly after the discovery of superior surface activities by Rosen and co-workers in the late eighties. Its surface performance can be ten to hundred times greater than its single parent surfactants (Cui & Canselier, 2000; Li *et al*., 1996). As compared to other binary mixed systems, anionic/cationic mixtures have the highest surface activities. Their oppositely charged head groups screen out the electrostatic repulsions between each surfactant molecules and enable close packing through ion-pairing.(Graciaa *et al*., 1989; Murphy and Taggart, 2002).

1.4 Catanionic Surfactants

Catanionic surfactants which have polar heads consisting counterions of the oppositely charged has been acknowledged as one of this new class of surfactants. The term ‘catanionic’ is the contraction of CATionic and ANionic surfactants. The differences between catanionic and anionic/cationic mixed surfactants are that catanionic surfactant is free of inorganic salt and the mixing ratios of anionic and cationic surfactants are exactly 1:1. Whereas in anionic/cationic mixtures, anionic/cationic complex coexists with inorganic salts and the mixing ratios of the oppositely charged surfactants can be varied (Hao and Hoffmann, 2004). Catanionic surfactants also differ very much from the classical zwitterionic surfactant. The counterion head groups in a catanionic surfactant are joined electrostatically where as zwitterionic surfactant’s counterion head

groups are joined covalently. Besides that, catanionic surfactant has two hydrocarbon tails while zwitterionic surfactant only has one. The differences between these surfactant systems are illustrated in Figure 1.2.

This co adjustment of electrostatic effects and surfactant molecular geometry allows a rich diversity of phase behaviour. Catanionic surfactants can self-assemble into various complex microstructures such as micelles, vesicles, lamellae, columnar and cubic mesophases, which are of far greater than exhibited by the individual and mixed surfactants. However, factors such as relative alkyl chain lengths, number of alkyl chains per surfactant, total surfactant concentration, surfactant mixing ratio, and temperature dictate the rich array of aggregates formed (Hao and Hoffmann, 2004; Khan and Marque`s, 1997). However, investigation on the micellization of a true catanionic surfactant has not been explored much, probably due to its low solubility in aqueous solution.



(a) (b) (c)

Figure 1.2 : Schematic diagram of (a) catanionic surfactant, (b) zwitterionic surfactant and (c) anionic/cationic mixed surfactants.

1.4.1 Formation of Catanionic Surfactants

Preparation of anionic/cationic mixtures is rather straight forward. Stock solutions of anionic and cationic surfactants were mixed at a desirable ratio and left to reach equilibrium which is usually very fast. The oppositely charged surfactant molecules spontaneously attract to each other via electrostatic interactions and consequently, form aggregations. As for catanionic surfactants, mixing ratios of anionic and cationic surfactants are always equimolar. Besides, additional process for removal of inorganic salt is required. There are 3 common ways of producing catanionic surfactants which are (1) precipitation follows by filteration, (2) solvent extraction and (3) conversion to its acid form by ion exchange resin (Rosen, 1989).

1.4.1.1 Precipitation Method

When an equimolar amount of the anionic and cationic surfactants in aqueous solutions are mixed at certain concentration, a precipitate will be yielded. The mixtures can then heated until clear liquid is obtained (addition of solvent may be needed for certain systems). The mixtures are slowly cooled to obtained crystalline precipitate of the catanionic surfactant. The precipitate is then filtered, washed several times to remove inorganic salt and unreacted ionic surfactants and finally dried under vacuum.

1.4.1.2 Solvent Extraction Method

Similar to the precipitation method, equimolar amount of anionic and cationic surfactants in aqueous solution is first prepared. Then, a suitable organic solvent was added in to extract the catanionic from the aqueous phase to the organic phase. The organic phase is then separated and evaporated under pressure to obtain the white crystals of the catanionic surfactant.

1.4.1.3 Ion Exchange Method

This method is quite different as compared to the methods discussed above. Anionic and cationic surfactants are dissolved in a suitable solvent (which is usually water). Then, the anionic surfactant is converted into its acidic form and cationic surfactant to its hydroxide form by eluting the surfactants through ion exchange columns which are packed with the appropriate ion-exchange resins. Purity of the surfactants can be enhanced by multiple elutions through the ion-exchange columns. The hydroxide is then titrated with the acid until neutral point is reached. Finally, the solid catanionic can be obtained by evaporating the solution under pressure.

1.5 Physical Properties

1.5.1 Critical Micelle Concentration (CMC)

As pointed out earlier, a surfactant molecule consists of a hydrophilic part and a hydrophobic part. When a surfactant is diffuse into an aqueous solution, the hydrophobic tail will move away from the water and reach out to the gaseous phase at the air/water interface. At the same time, the hydrophilic part of the surfactant molecules was also being pulled to the surface but remains in the aqueous phase. By adsorbing at the liquid/gas interface, these surfactants are in a state of lower energy as compared to molecules in the bulk. As the concentration of surfactant increases, surfactant molecules continue to fill up the space at the liquid/gas interface in the same manner. When the liquid/gas interface is saturated, surfactant molecules start to aggregate in the liquid phase, forming micelles where the hydrophobic tails are hidden inside the micelle core while the polar head groups are arranged at micelle surface. The point when the micelle start to form is known as critical micelle concentration (CMC). The different aggregation of surfactant molecules are showed in Figure 1.3.

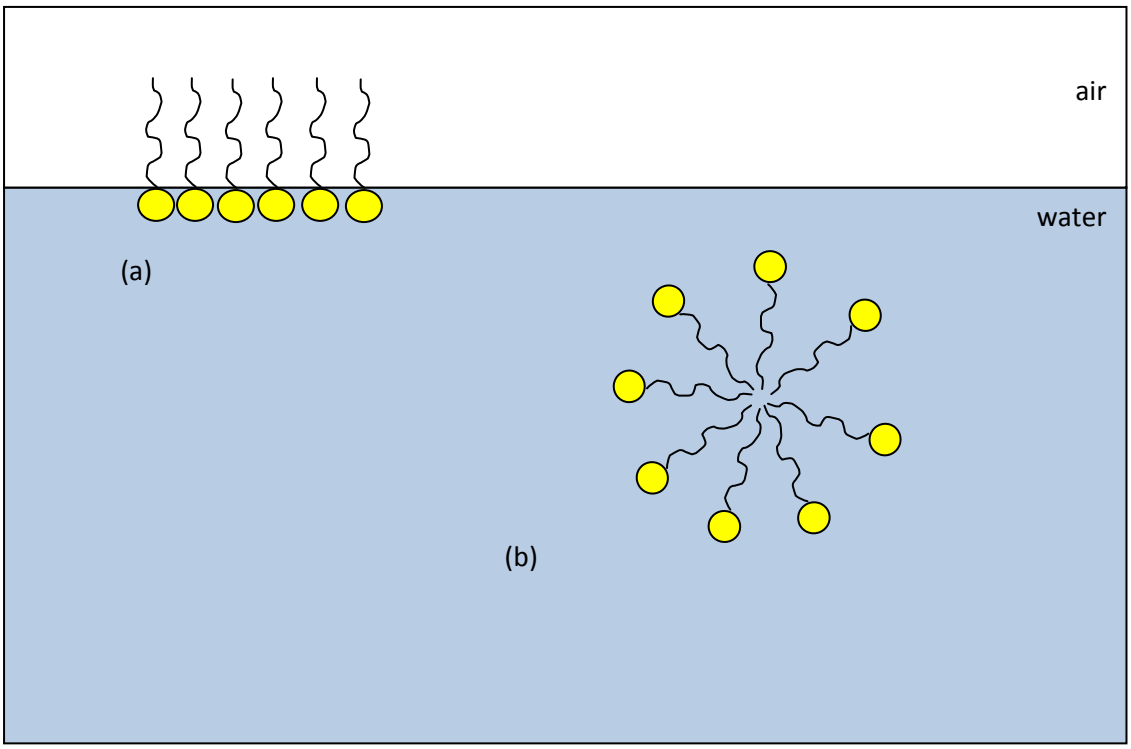


Figure 1.3 : Schematic illustration of surfactant aggregation as (a) liquid/gas interface monolayer and (b) micelle in aqueous solution.

Micelle formation is an important phenomenon that governs the interfacial phenomena of a surfactant system such as detergency, solubilization and interfacial tension reduction (Rosen, 1989). Many investigations have been concerned with determining values of CMC in various systems. These have lead to the discovery of a number of factors that affect the values of CMC; i.e. structure of the surfactant, presence of electrolyte, addition of organic materials, presence of a second liquid phase and temperature (Rosen, 1989).

Similar to single surfactant systems, mixed surfactant systems also undergo a series of micellization processes. It is well-known that mixtures of nonionic surfactants tend to mix ideally (Rosen, 1989), while mixtures of ionic-nonionic, anionic-cationic, and

hydrocarbon-based-fluorocarbon-based surfactants can deviate from the ideal behaviour (Rosen, 1989). Deviations from ideality result from either synergistic or antagonistic interactions between the different surfactant species. Synergistic interactions are favourable in many applications as it can have a lower CMC as compared to its single parent surfactants.

The superior performance of mixed surfactants and their unique behaviour over single surfactants are mainly due to their synergistic interactions between the different surfactant monomers in the mixed systems (Rosen, 1989). The degree of incompatibility between surfactant heads (e.g. anionic-cationic, anionic-gemini and cationic-nonionic) was reported to be directly correlated to the intensity of the synergistic interactions of the surfactant aggregations. Anionic/cationic mixtures exhibited exceptionally high surface activity due to their electrostatic interactions between the oppositely charged surfactants (Murphy and Taggart, 2002; Frese *et al*., 2003; Sohrabi *et al*., 2008; Tondre and Caillet, 2001; Li and Liu, 1994). These properties are useful in various industrial applications such as detergents, emulsifiers and dispersants (Murphy and Taggart, 2002; Tsuchiya *et al*., 2007; Lucassen Reynders, 1980).

In a work done by Vora and co-workers (1999), surface properties of different mixed systems (anionic-anionic, cationic-cationic and ionic-nonionic) were compared in terms of the critical micelle concentration values. Regular solution theory was employed for all the mixed systems. Interactions between the ionic-nonionic systems were found to be more significant as compared to the similarly charged surfactants. Results for cloud point and viscosity also agreed with this trend.

Graciaa *et al*. (1989) compared the critical micelle concentration of mixed micelles of anionic-nonionic, cationic-nonionic and anionic-cationic mixtures, where polyoxyethylene of varying chain length (POE) comprised the nonionic component, sodium dodecylbenzenesulphonate (SDBS) as the anionic component and tetradecyltrimethylammonium bromide (TTAB) as the cationic component. The synergistic interactions of the SDBS/TTAB were highest followed by SDBS/POE and then TTAB/POE. In addition, the large zone of synergism, the ternary mixed micelles would always compose of the equal number of anionic molecules and cationic molecules.

However, anionic/cationic mixtures have a tendency to form precipitates that renders it unsuitable for certain applications (Zhao and Li, 1990; Tomasic *et al*., 1991). Their solubility was reported to be governed by the type of head groups and symmetry of the tail groups (Li and Liu, 1994; Tomasic *et al*., 1991). For examples, a sulfonate head group in the anionic surfactant has a higher solubility as compared to sulfate head groups (Li *et al*., 2003) while high asymmetry in the tail groups results in higher solubility in the system (Silva and Marques, 2005; Silva *et al*., 2007). The mixing ratio affects the type of surfactant aggregations (Tomasic *et al*., 1991; Kume *et al*., 2008) and its effect on phase behaviour is more apparent in the dilute region where diverse microstructures are present (Li and Liu, 1994; Tomasic *et al*., 1991; Kume *et al*., 2008; Dragčević *et al*., 1995; O'Connor and Hatton, 1997).

In 2006, Šegota and co-workers investigated on the surface properties of sodium dodecylbenzenesulphonate (SDBS), didodecyldimethylammonium bromide (DDAB) and their mixtures by means of conductivity, tensiometry, potentiometry and electrophoresis. SDBS/DDAB mixtures showed a slightly lower critical micelle concentration (CMC) value as compared to the single surfactants.

Due to the tunability of catanionic surfactants, continuous studies on its physical properties are being carried out until today. Jiao *et al*. (2013) studied on a series of salt-free catanionic surface active ionic liquids (SAILs), 1-alkyl-3-methylimidazolim alkyl sulfates which were prepared by an ion exchange reaction. In addition to the improved surface activities of the catanionic surfactants, it was also observed that the self-assembly of these imidazolium-based salt-free catanionic SAILs can be tailored by adjusting the mismatch of alkyl chains.

1.5.2 Interactions between Surfactant Molecules

According to Rubingh's regular solution theory, interactions between the different surfactant molecules in the mixed systems can be measured by calculating the β parameter values from the plots of surface tension, γ, versus the concentration of aqueous solutions of the individual surfactants and at least one mixture of them. Since the value of β is proportional to the free energy of mixing of the systems, a negative value of β indicates attractive interactions between two different surfactant molecules was stronger than the attractive interactions between similar surfactant molecules and vice versa. β value close to zero shows that there are little or no interactions between the surfactant molecules which is commonly referred as ideal behaviour.

The β parameter for a mixed system can be calculated at the surface monolayer, β^{mon} and also in the micelles, β^{mic}. The value of β^{mon} is not the same as the value of β^{mic} for the same two surfactants under the same condition. For most mixed systems, the interaction in the mixed monolayer was greater than that in the mixed micelle (Sohrabi *et al*., 2008; Zhou and Rosen, 2003). Such behaviour is most sought after in the detergency industry as the strong synergistic interactions greatly enhance the surface properties of the surfactants (Sohrabi *et al*., 2008). However, there were cases where the interactions in the mixed micelle were stronger than the interactions in mixed monolayers (Sohrabi *et al*., 2008). Usually, interactions between the surfactant molecules were dominated by the hydrophilic part of the surfactants (Rosen, 1989; Sohrabi *et al*., 2008; Zhou and Rosen, 2003).

Interactions of dodecylammonium chloride (DDACl) – sodium dodecyl sulfate (SDS) mixtures at the air-water interface and equilibria in the bulk phase were investigated by Dragčević and co-workers (1995). Systems were prepared either with a constant DDACl and increasing SDS or vice versa. All measurements were carried out at 298K. The negative values obtained for the interaction parameter, β suggest synergistic forces between DDACl and SDS. The addition of an oppositely charged surfactant reduces repulsion and the minimum area per molecule decreases.

The effect on chain length compatibility on the micellar stability of the mixed system of sodium dodecyl sulfate (SDS) and alkyltrimethylammonium bromide (C_nTAB, n=8, 10, 12, 14, 16) was investigated by Patist *et al*. (1997). The surface properties of the mixed surfactants were observed to be depending on the chain length of the C_nTAB. Minimum surface tension, maximum surface viscosity, highest micellar stability, maximum foam stability and minimum foam ability were observed when both the surfactant chain

length is similar (SDS/ C_{12}TAB). SDS/ C_{12}TAB system also results in the closest packing of molecules in micelles and at the air/water interface.

Sohrabi *et al*. (2008) investigated the properties of anionic-rich and cationic-rich mixtures of cetyltrimethylammonium (CTAB) and sodium dodecyl sulphate (SDS). Strong synergistic interactions were found between the oppositely charged surfactants. The attractive forces in mixed aggregates were also found to be weaker than in a mixed monolayer at the air/solution interface. Besides that, the rise in temperature brings greater increase in the electrostatic interaction in anionic-rich mixtures than in cationic-rich mixtures. The effect of temperature is also found to be more prominent at the planar air/solution interface in an aqueous medium as compared to the convex aggregate surface.

In 2010, physicochemistry of the binary mixtures of cetylpyridinium chloride (CPC) and sodium dodecylsulfate (SDS) were investigated in detail by Maiti *et al*. Results were analyzed using Gibbs adsorption isotherm and the regular solution theory of Rubingh at five different CPC: SDS mole ratios. The equimolar composition was found to have the maximum interaction with a large synergistic effect. The isolated coarcervate was also characterized by means of FTIR, NMR, XRD and DSC which comfirmed the formation of ion-pair complex.

Interfacial behaviour of the myristic acid and cetyl trimethylammonium bromide (CTABr) catanionic mixtures were investigated by Stocco *et al*. (2010). The remarkable increase in foam stability of the catanionic as compared to CTABr was due to low surface tension, high surface concentration and high viscoelastic compression moduli of the mixed system. Foamability is related to the concentration of free surfactants and is

higher for nondialyzed samples: for solutions with f=0.35 that contain the largest amount of free CTABr, foamability is comparable to that of pure CTABr solutions. The rate of coarsening is similar for dialyzed and nondialyzed samples and much slower than for pure CTABr foams.

1.6 Phase Behaviour

The phase behaviour of aqueous surfactant system is far more complex and interesting as compared to non-surface active material, like salt. Besides existing in solid, liquid and gaseous phase, surfactants are also reported to self assemble into micelles, vesicles, lamellar phase, and liquid crystals. The aqueous behaviour of a surfactant is mainly influenced by its molecular structure but system variables such as temperature, pressure and composition also play a part (Laughlin, 1996).

1.6.1 Phase Behaviour of Catanionic Surfactants

Systems of this new class of catanionic surfactants also show very interesting phase behaviour, for example the formation of mixed micelles (of various shapes) at very low surfactant concentration, the presence of swollen lamellar phases, and spontaneous vesicle formation. This unique behaviour of catanionic surfactants or mixtures continues to fuel the enthusiasm of researchers around the world. Factors that affect the self-assembly behaviour of catanionic surfactants include total surfactant concentration, temperature, hydrophilic head group, hydrophobic chain length, presence of salt and so on.

From a fundamental point of view, catanionic systems display many interesting features. The richness and complexity of their aqueous phase behaviour permit qualitative discussion on the influence of electrostatics and molecular geometry on surfactant self

assembly. Catanionic mixtures have been shown to form various different types of surfactant aggregates, such as micelles of various forms and sizes, and lamellar structures, such as vesicles. These catanionic surfactants can exhibit novel solution and interfacial properties compared with those of mixed and parent surfactants. The aggregation in aqueous mixtures of catanionic surfactants occurs at considerably lower concentrations than the critical micelle concentration (CMC) of individual and other mixed (such as non-ionic-nonionic, cationic-nonionic) surfactants.

The catanionic amphiphiles systems have been investigated in the 1980s by Jokela and his co-workers (Jokela *et al*., 1987, 1988; Joensson *et al.*, 1991). In these systems, the initial inorganic counterions of the cationic and anionic surfactants have been replaced, respectively, by the hydroxide and hydronium ions by using ion exchange resins. In such systems, the polar head of each surfactant is actually the counterion of the oppositely charged surfactants. The removal of the low molecular weight salt is expected to modify the electrostatic interactions between the two surfactants by dramatically increasing the Debye screening length. As a result, the ion-pair interaction between the polar heads of oppositely charged surfactants are stronger than in catanionic systems with excess salt and this changes the total area occupied by the surfactants polar heads. Moreover, with salt-free catanionic surfactant solutions, there is no precipitation at equimolar concentrations as reported by Jokela et al. (1987).

Nakama *et al.* (1990) investigated the interactions between anionic and cationic surfactants by means of surface tension, conductivity and nuclear magnetic resonance. Due to the presence of strong electrostatic interactions, mixed surfactants showed superior surface properties as compared to the single surfactants. A phase diagram as a function of temperature for the water-stearyltrimethylammonium chloride (STAC)-

sodium laurate (NaL) system was also mapped out. The Krafft point rose remarkably around the equimolar mixing ratio, which suggests the formation of equimolar complex. Whereas, the phase diagram of water- stearyltrimethylammoniumchloride (STAC)-sodium-N-lauroyl-N-methyl-β-alanine (NaLMA) system showed that the Krafft point changes monotonously with the change in composition of mixed surfactant (Nakama *et al.*, 1990). The most striking characteristic is that the one-phase region is very wide for STAC-NaLMA system as compared to that of the general nonionic surfactant.

The phase diagram of sodium dodecyl sulfate (SDS)-didodecyldimethylammonium bromide (DDAB)-water system at 40 ^{o}C was studied by Marques *et al.* (1993). The appropriate amounts of SDS, DDAB and water were weighed in glass tubes and flame-sealed. The samples were also centrifuged at certain time intervals to attain equilibration. There is a large region of bicontinuous cubic liquid crystalline phase, with high concentrations of both surfactants. The phase diagram contains several regions of lamellar liquid crystalline phase, and under certain conditions two lamellar phases may coexist. Formation of vesicles was only found in water rich regions.

In the work of Chen *et al.* (2004), they highlighted the striking difference between alkyl sulfate (C_nSO_4; n=8, 10, 12) and alkyl sulfonate (C_nSO_3; n=8, 10, 12) when mixed with alkltrimethyl/ethyl/butylammonium bromide (C_nNM, C_nNE and C_nNB). In contrast to the single surfactant, C_nSO_3-C_nN mixtures were much more soluble than C_nSO_4-C_nN mixtures. Their catanionic surfactant mixtures were also quite different in solution properties, phase behaviour and aggregate properties. This major difference in the catanionic surfactants is attributed to the different molecular charge distribution of alkyl sulfate and alkyl sulfonate.

A successful control in size and shape of aggregates of a catanionic system was realized by Zemb and co-workers (Dubois *et al*., 1998, 2001, 2004; Zemb *et al*., 1999; Glinel *et al*., 2004). The model system used was composed of cetyltrimethylammonium hydroxide and myristic acid, mixed at a total surfactant concentration of 20 g/L. Along variation of the mixing ratios, the surfactant systems led to the formation of different aggregate structures, such as nanodisks or icosahedra. The catanionic mixtures are first warmed up. The initial states of the dispersion at temperatures above 50^{o}C are generally composed of unilamellar vesicles in the fluid state. Upon cooling, nucleation and growth of planar crystals occur in the form of frozen bilayers. Both nanodisks (Dubois *et al*., 1998, 1999) and facetted objects, such as icosahedra (Dubois *et al*., 2001) assemble to form frozen aggregates. When the water insoluble anionic component is in excess, micrometer-sized icosahedra are formed. For catanionic solutions in an excess of anionic component, icosahedra are formed by evacuation of the excess charges which are segregated into pores. The presence of insoluble excess fatty acid produces pores at vertices. With a slight excess of water soluble cationic component, the surfactants will aggregate into nanodisks which are sandwich-like microstructures. Nanodisks are thus formed by rejection of excess charges towards the edges.

The influence of the polar head group on the phase behaviour of catanionic surfactants were studied in depth by Marques and his co-workers (Silva and Marques, 2005; Marques *et al.*, 2006). The thermotropic behaviour was studied by DSC and the mesophase structures were investigated using a polarizing microscope. Six types of catanionic surfactants namely hexadecylpyridinium octylsulfate (PS), hexadecyl pyridinium octylsulfonate (PSo), hexadecylpyridinium 4-octylbenzenesulfonate (PBSo), hexadecyltrimethylammonium octylsulfate (TAS), hexadecyltrimethylammonium octylsulfonate (TASo) and hexadecyltrimethylammonium 4-octylbenzenesulfonate

(TABSo) were prepared and compared in the work of Silva and Marques (2005). The phase behaviour of the TA-series is more complex than the P-series. The charge density of the polar head group seems to influence the transition temperature, whereas packing effects were more dependable on the electrostatic effects. Whereas in the work of Marques *et al.* (2006), catanionic surfactants were prepared from dioctadecyldimethylammonium bromide ($2C_{18}C_{12}$) with varying anionic surfactants; octylsulfate (C_8S), decylsulfate ($C_{10}S$), dodecylsulfate ($C_{12}S$), octylsulfonate (C_8So), benzenesulfonate (BzSo), decylcarboxylate ($C_{10}CX$). All the investigated triple-chained catanionic surfactant undergoes transition from the solid crystalline to the isotropic liquid, namely solid-solid, solid-lc, and lc-lc phase transitions upon heating. Lower asymmetry in chain length and higher head group charge density generally induce higher number of phase transitions, higher transition temperature and enthalpy values. Matos *et al.* (2013) continue to study on the thermotropic phase behaviour of catanionic surfactants until today. Three series of compounds, namely: the $TA_{16}So_n$ series, where n = 6–10; the TA_mSo_8 series, where m = 12–16; and a constant m + n series, TA_mSo_n where m + n = 22 were investigated by Matos *et al.* (2013). Phase transition temperatures and transition enthalpies/entropies were determined by differential scanning calorimetry, while mesophases were assigned by polarized light microscopy. The study reported that chain length mismatch in catanionic surfactants induces rich mesomorphism. Solid phase stability was enhanced with higher chain asymmetry meanwhile lower asymmetry promotes the stability of smectic liquid crystals.

Another interesting study was also carried out by Tah *et al.* (2011) on the aggregation behaviour of catanionic mixtures of the anionic surfactant sodium dodecyl sulfate (SDS) and the cationic surfactant cetyltrimethylammonium bromide (CTAB) in solution and at the air/water interface by using the Langmuir-Blodgett (LB) technique. Fourier

transform infrared spectroscopy, *in situ* phase-contrast inverted microscopy, scanning electron microscopy, and atomic force microscopy were also used to characterize the catanionic surfactants in solution, air/water interface and LB film. Formation of mixed micelles was observed at the air/water interface with SDS/CTAB ratios in the range from 35/65 to 65/35. Meanwhile in the aqueous solution, spherical vesicles were formed in the same compositions. For the remaining compositions (90/10, 10/90), unorganized aggregations were observed.

1.6.2 Formation of Stable Vesicles

Of particular interest in catanionic mixtures is the formation of vesicular phases, which are not observed when either surfactant is mixed alone with water. Unlike the metastable unilamellar vesicles formed from biological lipids, catanionic surfactant vesicles can be thermodynamically stable. Similar to other surfactant systems, aggregation behaviour of catanionic surfactants is governed by hydrophobicity and packing geometry (Israelachvili, 1977). The oppositely charged head groups of the catanionic surfactants act as a counterion to each other and attractive forces between the neighbouring surfactant molecules are greatly strengthen. Hence, the surface area of the particular surfactant system is reduced. Such packing geometry favours curved bilayers instead of planar bilayers as less energy is needed (Hoffmann, 1999)

Fukuda *et al.* (1990) investigated various catanionic systems composed of alkylammonium hydroxide and alkylcarboxylic acid with equal chain lengths of C14, C16 and C18. All catanionic systems proved to form vesicles at stoichiometric quantities but the structures formed showed only a short-term stability (from 1h to maximum 48h). The stability is very different for vesicles formed from mixed catanionic surfactants systems with excess salt. Catanionic vesicles are in this case

stable for periods as long as several years, since they appear to be the equilibrium form of aggregation.

In 1992, Kaler and his co-workers found that spontaneous, single-walled, equilibrium vesicles of controlled size and surface charge can be formed by aqueous mixtures of anionic and cationic surfactants. They specifically studied the catanionic mixtures of sodium dodecylbenzenesulfonate (SDBS) and cetyl trimethylammonium tosylate (CTAT). The regions of stable vesicles that mimic the double-tailed phospholipids in the phase diagram are bounded by lamellar and micellar phases. The superior stability of the catanionic vesicles as compared to typical phospholipids are due to electrostatic interactions and enhanced steric hydration forces. These catanionic vesicles are stable for periods as long as several years and appear to be the equilibrium form of aggregation (Kaler et al., 1992).

The effect of electrostatics on the phase behaviour of cetyltrimethylammonium bromide (CTAB) and sodium octyl sulphate (SOS) mixtures with added sodium bromide (NaBr) was studied by Brasher *et al.* (1995). An obvious change was observed when NaBr was added into the catanionic mixtures. At certain composition, the added salt induced a transition from vesicles to micelles. From this study, a thermodynamic cell model has been developed to foresee the phase behaviour and related properties of a mixed system.

Hao *et al.* (2003) investigated the catanionic systems composed of tetradecyltrimethylammonium hydroxide ($C_{14}N(CH_3)_3^+OH^-$) as the cationic component with different anionic surfactants of various chain lengths. Only the cationic high ratio side of the phase diagram was investigated, since the fatty acids are insoluble in water at

25^{o}C. The systems were first heated up to 70^{o}C to be homogenized and cooled down afterwards to 25^{o}C. The formation of polydisperse vesicular solutions with diameters ranging from 30 nm to 200 nm could be observed in all systems close to equimolarity. The only difference when changing the chain length of the anionic surfactant was that the longer the chain length, the closer the existence range of the vesicular phase would start from equimolarity.

Comparison between the phase behaviour of zwitanionic and catanionic mixtures of perfluoro and hydrocarbon surfactants were done by Wolf *et al.* (2009), where lithium perfluorooctanoate (LiPFO) as anionic surfactant and tetradecyldimethylamine oxide (TDMAO) as zwitterionic or tetradecyltrimethyl ammonium bromide (TTAB) as cationic surfactant. Despite the equal chain length of TDMAO and TTAB, the phase diagram is largely different. A larger region of stable unilamellar vesicles was observed in the LiPFO-TDMAO system. Meanwhile, larger vesicles were formed in the LiPFO-TTAB system which have stronger tendency to form multilamellar vesicles. From this study, it was found that the structure and stability of the vesicles were highly dependable on the electrostatic interactions.

In the work of Zhao *et al.* (2012), spontaneous formation of vesicles by catanionic system composing of a cationic surface active long-chain ionic liquid N-dodecyl-N-methylpyrrolidinium bromide (C_{12}MPB) and an anionic surfactant sodium dodecyl sulfate (SDS) was investigated. The phase behavior of the catanionic system is studied and the phase diagram is mapped through visual observation and electrical conductivity measurement. The hydrophobic and electrostatic interactions are found to be the driving forces for spontaneous formation of vesicles.

In addition, the ability to form reverse vesicles spontaneously by the catanionic system of tetradecyltrimethyl ammonium laurate (TTAL) and lauric acid (LA) in toluene was also discovered by Li *et al.* (2013). The reverse vesicular phase is found to be quite stable and can be labelled with fluorescent dyes for confocal fluorescence microscopy observations. Besides, cryo-TEM observations were also applied to probe the morphology of the reverse vesicles. With extended observation time, interesting intermediate structures were observed including onions, sheets and cellular networks.

1.7 Biological Properties

Quaternary ammonium compounds (QACs) are cationic surfactants that have been long known for its broad spectra of antimicrobial activities (Shimizu *et al*., 2002), especially in inhibiting the growth of Gram-positive bacteria (Sanchez *et al*., 1994). As such, the use of QAC as disinfectant in the medical care, food industry, detergent and glue industries are very common since its discoveries in the early 1900 (Sütterlin *et al.*, 2008). Extensive studies have been done on QACs of varying chain length and chemical structures (Balgavý and Devínsky, 1996; Salton, 1968; Valko and Dubois, 1945). It was reviewed that antimicrobial actions generally begin with penetration into the cell wall, follow by partitioning of the agents in between the core membrane, solubilizing the membrane and then lysing the cells. Death of the cells occurs through leakage of cytoplasmic materials (Salton, 1968; McDonnell and Denver Russell, 1999). Beside this, there are also many other different mechanisms employed by QACs to cause lethality to the microbes depending on the nature of the living organisms (McDonnell and Denver Russell, 1999). For example, QACs are able to induce the leakage of K+ and pentose material from the yeast S. *cerevisiae* and induces protoplast lysis as well as interacting with crude cell sap (Hiom *et al*., 1993). In another study, QACs were also found to

induce disintegration and morphological changes on enveloped viruses (Prince *et al*., 1993).

Anionic/cationic mixtures or catanionic surfactants might possess a certain degree of anti-microbial properties. Both surfactant systems are amphiphilic in nature, therefore they have a high tendency to interact with the cell membranes. Such interactions may lead to the death of microbes. Other factors that contribute to this characteristic are the presence of quarternary ammonium compound in the cationic component of the anionic/cationic mixtures or catanionic surfactants which has anti-microbial properties (Sütterlin *et al.,* 2008).

Little work has been undertaken to investigate on the efficiency of catanionic surfactants to inhibit the growth of bacteria or other microbes. The closest related work would be the one conducted by Sütterlin *et al.* (2008). The study investigated the effect of adding anionic surfactants such as linear alkylbenzene sulfonate (LAS), naphthalene sulfonic acid (NSA), sodium dodecylsulfonate (SDS), and benzene sulfonic acid (BSA) into the cationic quaternary ammonium compound benzalkonium chloride (BAC). Inhibitory activity of such mixtures at various mixing ratios against *Pseudomonas putida* and *Vibrio fischeri* were tested. Results showed that the addition of anionic surfactants do not render the BAC useless. In certain systems, improvement of inhibitory activity was observed (Sütterlin *et al.,* 2008).

On the other hand, there was an interesting study that investigated the mechanism of interaction between catanionic vesicles and cell membranes (Boudier *et al.,* 2011). The toxicity of tricatenar catanionic surfactant 1-N-hexadecylammonium-1-deoxylactitol-bis(R-hydroxydodecylphosphinate), so-called “TriCat” were checked on peripheral

blood mononuclear cells (PBMCs) and macrophages. Results showed that catanionic surfactants interact with the cell membrane by membrane fusion instead of monomeric partitioning into membrane cell.

1.8 Application of Catanionic Surfactants

The closed structures spontaneously formed in catanionic systems represent a possible novel route for encapsulation. Active molecules can be encapsulated in the bilayer membrane if they are lipophilic or in the core of the vesicle if they are hydrophilic. Encapsulation is useful to protect actives in preventing any undesired reaction such as oxidation (Ostro, 1987).Vesicles can thus be used as vectors to deliver drugs to a specific place, without them being destroyed during the delivery.

The first encapsulation experiments were performed by Hargreaves and Deamer (1978) on the cetyltrimethylammonium bromide/ sodium dodecylsulfate system. The system was heated up to 47^{o}C but the vesicles observed at this temperature appeared impermeable to sucrose. At lower temperatures, the vesicle degenerated into angular membrane fragments. Temperature appears therefore a predominant parameter for the ability of catanionic vesicles to encapsulate actives.

Fischer *et al.* (2002) proceeded to perform glucose entrapment experiments from vesicles formed of cetyltrimethylammonium tosylate and sodium dodecylbenzenesulfonate (CTAT/SDBS) mixtures. Vesicles formed in the presence of glucose were equal in size to those formed in pure water. No further quantitative detail concerning the entrapment rate of glucose was mentioned but vesicles appeared to encapsulate and retain glucose which are determined by dialysis method. A more comprehensive study of the entrapment ability of the SDBS/CTAT system was

conducted by Tondre *et al.* (2001). The CTAT-rich vesicles appeared less efficient as regards entrapment as the SDBS-rich vesicles. The overall surfactant weight percentage appeared to have a strong influence as well on the encapsulation ratio, since increasing the total surfactant concentration from 0.5wt% to 2.5wt% for SDBS-rich vesicular systems lead to an increase in the encapsulation ratio from 0.5 to 3%.

In a different study, the ability of the catanionic system didodecyltrimethyl ammonium bromide/ sodium dodecylsulfate (DDAB/SDS) to encapsulate glucose was investigated by Kondo *et al.* (1995). The separation of free end entrapped glucose was achieved through dialysis experiments. Addition of Triton X- 100 (non-ionic surfactants being known to disrupt the membranes) was necessary to induce the release of glucose. The maximum encapsulation percentage reached was 7.9%.

Another possibility for encapsulating a drug in a catanionic vesicle is to use a charged drug as one of the components of the cationic / anionic mixture and induce a controlled release of the drug in using a gel as a vehicle (Paulsson and Edsman, 2001). Bramer *et al.* (2003) studied a mixture of SDS and positively charged drugs such as diphenhydramine, tetracaine or amitryptiline. Such systems formed the same interesting phases as traditional catanionic mixtures. A Carbopol or Agar gel containing the vesicles was used as a drug carrier and proved to be useful in obtaining functional controlled-release systems. Using the same concept, Zhao and co-workers (2013) did a much complete study on the feasibility of catanionic vesicles as a safe drug delivery vehicle. The catanionic complexes formed by an active drug (diclofenac sodium, DS) and conventional surfactant (didodecyldimethyl ammonium bromide, DDAB) are investigated. It was found that both the drug release behavior and the hemolytic toxicity are dependent on the composition of DS in the samples. In conclusion, the study

suggested that the drug-participating catanionic vesicles can be used as a safe and an efficient vehicle for sustained drug release.

Drug delivery mechanism of catanionic vesicles 1-N-hexadecylammonium-1-deoxylactitol-bis(R-hydroxydodecylphosphinate), abbreviated as "TriCat", was explored by Castagnos and co-workers (2010). The interaction modelled with giant liposomes as membrane models confirmed the occurrence of a fusion phenomenon between the nanovectors and the cell membranes of phagocytic and non-phagocytic cells. This process highlights the potential of catanionic vesicles for a future pharmaceutical application as a universal drug delivery system.

In a recent work by Liu *et al.* (2013), a catanionic based carrier for enhanced skin delivery of drugs is discovered. The catanionic system formed by decyltrimethylammonium bromide (DeTMAB) and sodium dodecyl sulfate (SDS) were prepared via precipitation method with the aid of ethanol as the cosolvent in aqueous buffer solution by a simple semi-spontaneous process. The potential application of the catanionic vesicles as nano-carriers in dermal drug delivery was demonstrated by the encapsulation of vitamin E acetate (α-tocopherol acetate, α-TA). It was reported that the encapsulation efficiency of α-TA in the catanionic vesicles is dependent on the membrane rigidity of the vesicle, which can be tailored by the addition of cholesterol.

Apart from its popular function as a delivery vehicle, Akong and co-workers (2013) successfully applied catanionic surfactants in formulation of boron wood-preservatives. The new catanionic consisting of amphiphilic carnosine (βAlaHisC8) and lauric acid forms supramolecular hydrogel at a very low concentration. By adding a mere 0.3% of the gelator agent (w/w), the resistance of Scots pine sapwood subjected to water

leaching toward the brown-rot fungus *Poria placenta* was greatly improved. These results clearly indicate the effectiveness of hydrogel to retain boron in wood.

In another study by Khurana *et al.* (2010), catanionic surfactanst which are synthesized by taking equimolar mixture of acid (hexanoic acid, octanoic acid and stearic acid) and hexylamine are investigated. Three new microemulsions were prepared using the catanionic surfactant systems. Decrease in the water-intake capacity of the microemulsions with increase in the chain length of the acid part of the catanionic surfactant was reported. These microemulsions were found to be suitable for the formation of quantum dots of Cadmium Sulfide (CdS) with size varying from 3 to 5 nm as calculated from spectroscopic studies. It was also showed in the study that fine adjustments in the size of CdS quantum dots are possible by varying the chain length of the acid moiety.

1.9 Objectives of Present Study

The aim of the present study is to synthesize different alkyl chain lengths of alkyltrimethylammonium-alkylmethylestersulfonate palm catanionic surfactants. The alkyltrimethylammonium (C_nTAB) is a conventional cationic surfactant, sodium alkyl methyl ester sulfonate (C_mMES) is a relative new anionic surfactant that has considerably good surface activities as compared to conventional anionic surfactants such as alkyl sulfate (AS) and linear alkylbenzene sulfonates (LAS). The C_mMES also exhibits better biodegradability as compared to other anionic surfactants of its grade. The present study on the physicochemical properties of C_mMES.C_nTAB catanionic as well as C_mMES/C_nTAB mixtures were presented. Their micro- and macroscopic self-assembly properties especially the new catanionic ion-pair in aqueous solution provide possible identification of potential applications of the novel catanionic surfactant systems as a carrier for active ingredients.

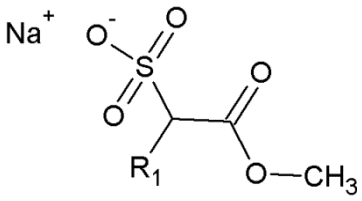
In addition to its role as cationic component in the mixed systems, C_nTAB also double-up as an active ingredient with bacteriostatic properties. Increase in interfacial activities also further enhances inhibitive properties of the mixed systems. Thus, anionic/cationic mixtures and catanionic surfactant systems were also evaluated for their biological properties by means of minimum inhibition concentration (MIC) assay. Both Gram-positive and Gram-negative bacteria were tested.

CHAPTER TWO

EXPERIMENTAL DESIGN

2.1 Materials

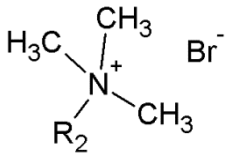
Cationic surfactants, namely, dodecyltrimethylammonium bromide (C_{12}TAB), tetradecyltrimethylammonium bromide (C_{14}TAB) hexadecyltrimethylammonium bromide (C_{16}TAB) and octadecyltrimethylammonium bromide (C_{18}TAB) were purchased from Sigma-Aldrich, Switzerland. All alkyltrimethylammonium bromide surfactants were recrystallized three times with acetone (Sigma-Aldrich, Switzerland) before use. Sodium dodecyl methyl ester sulfonate (C_{12}MES), sodium tetradecyl methyl ester sulfonate (C_{14}MES) and sodium hexadecyl methyl ester sulfonate (C_{16}MES) were obtained from Malaysian Palm Oil Board (MPOB). Purification of MES involved distillation with methanol (Sigma-Aldrich, Switzerland) to remove disalt of MES, followed by filtration to remove any impurities and finally vacuum dried at 60˚C to remove water and solvent. As the surfactants are hygroscopic in nature, all samples were sealed with parafilm and kept under vacuum in a dessicator until further use. All single surfactants did not show any minimum value in the surface tension versus concentration curve which confirmed high purity of the surfactant samples. Chemical structures of C_mMES and C_nTAB are shown in Figure 2.1.



C_mMES

$R_1 = C_mH_{2m+1,}$

where m= 12, 14, 16



C_nTAB

$R_2 = C_nH_{2n+1,}$

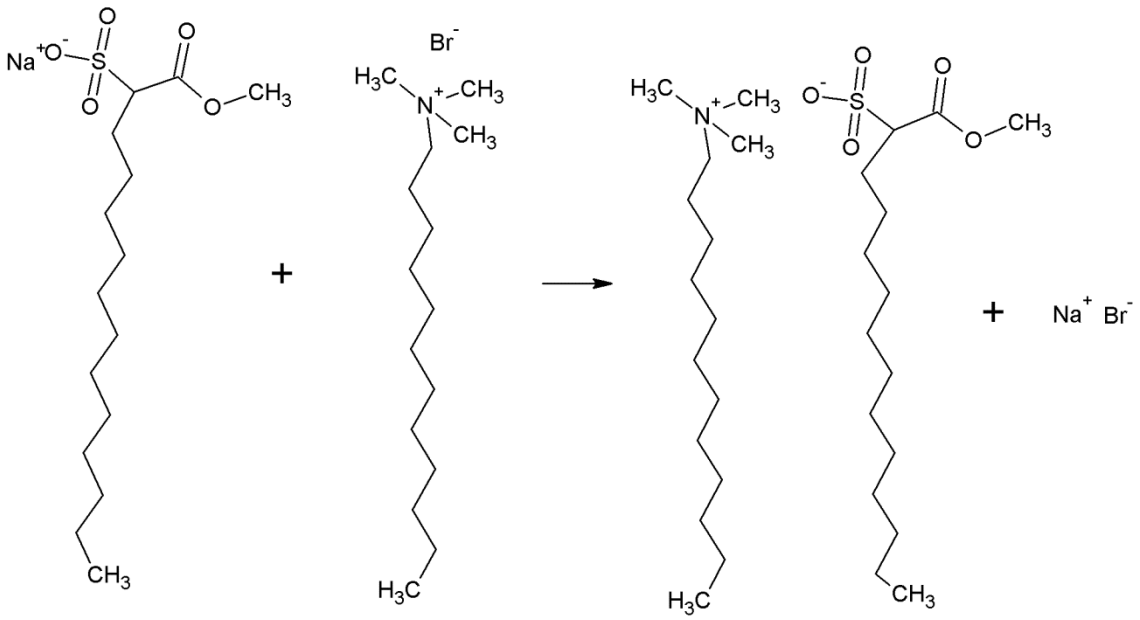
where n= 12, 14, 16, 18

Figure 2.1 : Structures of sodium methyl ester α-sulfoalkylate and alkyl trimethylammonium bromide.

2.2 Formation of Catanionic Surfactants

2.2.1 Preparation of Catanionic Mixtures

Firstly, all single surfactants (C_mMES and C_nTAB) were prepared in a stock solution at 25mM. Approximately 0.75-1.00 g of solid samples were weighed and added into 100 mL of distilled water. Solids were gently swirled to dissolve in a 100 mL volumetric flask to avoid formation of bubbles. Catanionic surfactants were then prepared by mixing the stock solution of two surfactants ($C_mMES.C_nTAB$) at 1:1 molar ratio, where m= 12, 14, 16 and n= 12, 14, 16, 18 carbon number in the hydrophobic chain. C_mMES was added drop wise into solution of C_nTAB and the catanionic mixtures were gently swirled for 5 minutes. This is followed by placing the catanionic solutions in water bath at 35˚C for 72 hours to equilibrate. Formation of catanionic mixed system is illustrated in Figure 2.2.



C_mMES C_nTAB C_mMES.C_nTAB

Figure 2.2 : Association between C_mMES and C_nTAB surfactants to form C_mMES.C_nTAB catanionic surfactants.

2.2.2 Optimization of Catanionic Surfactants to form Precipitates

In order to obtain a pure catanionic surfactant, inorganic salts (NaBr) and unassociated single surfactants need to be removed *via* a filtration method. Therefore, it is important to make sure that the catanionic surfactants aggregate into precipitates to ease the filtration process. As encountered in numerous trials, C_mMES.C_nTAB catanionic surfactants have the tendency to form aqueous two phase system (ATPS) which are impossible to filter. There are many factors that govern the aggregation behaviour of the catanionic surfactants, such as total surfactant concentration, mixing ratios of anionic and cationic surfactants and temperature. In this case, concentrations of the catanionic surfactant systems play the main role in determining the aggregation behaviour as the mixing ratio of anionic and cationic surfactants is fixed.

Figure 2.3 : Example of $C_mMES.C_nTAB$ catanionic precipitates that are ready to be filtered.

Each catanionic mixture was prepared in a series of concentrations (0.5mM, 1.25mM, 2.5mM, 5mM and 12.5mM) in test tubes and left to equilibrate at 35˚C for 48 hours. Then, appearance of the catanionic solutions at different concentrations was observed. A sheer cloudy solution shows that the concentration is too low and the aggregates are mostly micelles and vesicles. Meanwhile, thick and viscous white solution shows formation of an ATPS and indicated that the concentration is too high. The aim for this test was to obtain an almost clear solution with a white precipitate that deposited at the bottom of the test tube (Figure 2.3). For certain systems, catanionic solutions needed to be cooled down to 5˚C overnight in order to obtain precipitates. From our investigation, we found that $C_{12}MES.C_{14}TAB$ and $C_{12}MES.C_{16}TAB$ catanionic series formed precipitates at 2.5 mM while $C_{12}MES.C_{12}TAB$ and $C_{12}MES.C_{18}TAB$ system formed precipitates at 1.25mM. Most of $C_{14}MES.C_nTAB$ catanionic series formed precipitates at 1.25mM except for $C_{14}MES.C_{18}TAB$ system which formed precipitates at 2.5 mM.

C_{16}MES.C_nTAB catanionic series were also forming precipitates at 1.25mM except for C_{16}MES.C_{18}TAB system which is only able to form precipitates at 0.5mM.

2.2.3 Filtration of Catanionic Precipitates

After equilibration, the catanionic solutions were frozen at 0˚C in the freezer compartment of a conventiona Fridge (Panasonic NR-BK346MS, Japan) to enhance the formation of precipitates. After thawing, catanionic solutions were filtered using a cellulose nitrate membrane filter (MF-Millipore, USA) of 0.2 μm pore size in cold condition (below 5˚C). Catanionic surfactants were washed copiously with cool distilled water several times to remove impurities such as NaBr salts, sodium methylate sulfate (SMS) and any unreacted single surfactants. Lastly, catanionic surfactants were vacuum-dried overnight. In some cases, acetone was added into the catanionic solutions, which were then recrystallised and filtered to obtain the catanionic sample. Systems that need this extra step include C_{12}MES.C_{18}TAB, C_{14}MES.C_{12}TAB and C_{16}MES.C_{12}TAB. The final appearance of the catanionic surfactants are in the form of white solid.

2.3 Characterization of Catanionic Surfactants

2.3.1 Nuclear magnetic resonance (NMR) spectroscopy analysis

2.3.1.1 ^{1}H NMR

^{1}H NMR measurements of the catanionic surfactants as well as C_nTAB were recorded in a Bruker Avance 400 (Rheinstetten, Germany), FT-NMR spectrometer at 400MHz. The pulse width used was typically 6.95 μsec (90 °) and the pulses are repeated at 6.91 sec intervals. The 32K data points were used at a sweep width of 7994 Hz and a line broadening constant of 0.2 Hz. All ^{1}H spectra required about 8 scans (5 min).

All samples were run in 5 mm tubes employing deuterated chloroform, $CDCl_3$ as solvent and for deuterium lock. About 5 mg of solid samples were weighed into 0.5 mL of $CDCl_3$. The final concentration of the samples were 1 wt%. All samples were prepared freshly prior to measurements to avoid aging complications. The chemical shifts were referenced to tetramethysilane (TMS).

2.3.1.2 ^{13}C NMR

^{13}C NMR measurements of C_nTAB and the catanionic surfactants were recorded in a Bruker Avance 400 (Rheinstetten, Germany), FT-NMR spectrometer at 125MHz. The pulse width used was 4.2 μsec (30°) and repeated at 5.3 sec intervals. The 32K data points were used at a sweep width of 18307 Hz and a line broadening constant of 0.2 Hz. About 1000 scans are required for determination of peaks as low as 1%.

All samples were run in 5 mm tubes employing deuterated chloroform, $CDCl_3$ as solvent and for deuterium lock. As the sensitivity of ^{13}C is 6000 times lower than ^{1}H, concentrations of the samples were prepared as high as possible depending on their solubility. C_{12}MES.C_nTAB catanionic surfactant series have the highest solubility and were prepared at 10 wt%. C_{14}MES.C_nTAB catanionic surfactant series were also prepared at 10 wt% except for C_{14}MES.C_{18}TAB system which has a lower solubility and was prepared at 5 wt%. The remaining C_{16}MES.C_nTAB catanionic surfactant series were prepared at 5 wt%. All samples were prepared fresh prior to measurements to avoid aging complications. The chemical shifts were referenced to tetramethysilane (TMS).

2.3.2 Fourier Transform Infrared (FTIR) Analysis

The Fourier transform infra red (FTIR) spectra of the precursors and catanionic surfactants were taken using Magna-IR 550, Nicolet spectrophotometer (USA) in the range of 4000–450cm^{-1}. The resolution of the spectra was 5 cm^{-1}. Approximately 1.0 wt% sample is well mixed into 200 to 250 mg potassium bromide (KBr) powder and then finely pulverized and put into a 13 mm pellet-forming die. A force of approximately 5000-10000 psi is applied for several minutes to form transparent pellets. If it is translucent, the sample mixture will be reground and repressed. All spectra were scanned 16 times.

2.3.3 Polarized Light Microscopy Analysis

The morphology and texture of the solid crystalline phases of catanionic surfactants were determined under a polarizing microscope (Nikon ECLIPSE LV100POL, Japan) equipped with a camera (evolution TMVF cooled colour media cybernetics). The coacervate was dispersed in distilled water at a concentration of 1.0 mgmL^{-1}. One drop of the solution was spread on a cleaned glass slide and images were taken under bright field followed by polarized light using cross-polarizing filters.

2.4 Physical Properties

2.4.1 Mixed Anionic/Cationic Surfactants

2.4.1.1 Preparation of Mixed Anionic/Cationic Surfactant Solution-

All single surfactants were prepared in a stock solution with concentrations ranging from 1 mM to 40 mM. Mixed surfactants were then prepared by mixing the stock solution of two surfactants according to the desired molar ratio (C_mMES/C_nTAB) i.e; 5:95, 20:80, 40:60, 60:40, 80:20 and 95:5, where m= 12, 14, 16 and n= 12, 14, 16, 18 carbon number in the hydrophobic chain. C_mMES was added drop wise into solution of

C_nTAB and the mixtures were gently swirled for 5 minutes. The mixed solutions were kept for 24 hours in a water bath at 30˚C before performing surface tension measurements except for the C_{16}MES/C_nTAB and C_mMES/ C_{18}TAB mixtures which were kept at 35˚C.

The concentration of the stock surfactant solution played an important role in obtaining a good surface tension curve for analysis. If the concentration prepared was too high, there will be insufficient points to determine the onset of critical micelle concentration (CMC) accurately. If the concentration is too low, the plots will not reach the CMC breakpoint or the plateau region will be too short for determining CMC. Stock solutions for single surfactants are prepared according to the CMC values referenced from previous studies (Murphy and Taggart, 2002; Sohrabi *et al*., 2008; Satsuki, 1998). As for the mixed surfactants, a stock solution at 5 mM was prepared and a test run was conducted. After obtaining an approximate value of CMC, the concentration of the stock solution was refined accordingly.

From our experiment, C_{12}MES/C_{12}TAB and C_{12}MES/C_{14}TAB mixtures at all mixing ratios were prepared at 2 mM while C_{12}MES/C_{16}TAB and C_{12}MES/C_{18}TAB systems were prepared at 1 mM. Preparation for C_{14}MES/C_nTAB mixtures was more tedious as the CMC values change drastically from one system to another. C_{14}MES/C_{12}TAB and C_{14}MES/C_{14}TAB systems were prepared at 1 mM except for the mixing ratios of 0.05:0.95 and 0.95:0.05 which were prepared at 2 mM. In a similar trend, concentration for C_{14}MES/C_{16}TAB and C_{14}MES/C_{18}TAB systems with 0.05:0.95 and 0.95:0.05 mixing ratios were prepared at 1 mM while the rest were prepared at a lower concentration which is 0.5 mM.

Both C_{16}MES/C_{12}TAB and C_{16}MES/C_{16}TAB mixed systems at all mixing ratios were prepared at 0.2 mM. As for C_{16}MES/C_{18}TAB mixtures, 0.05:0.95, 0.80:0.20 and 0.95:0.05 mixing ratios systems were prepared at 1 mM while the rest were prepared at 0.2 mM.

2.4.1.2 Surface Tension Measurement (CMC)

The critical micelle concentration (CMC) for all single surfactants and mixed surfactants was determined by the surface tension (γ) measurement using a du Nouy ring tensiometer (KSV Sigma 70, Helsinki). Surface tension measurements of single surfactants and binary mixtures of C_{12}TAB, C_{14}TAB, C_{16}TAB, C_{12}MES and C_{14}MES were tested at 25±0.1˚C whereas C_{18}TAB, C_{16}MES and their mixtures were conducted at 35±0.1˚C. The concentration of the surfactant was varied by dilution of the stock surfactant solution with water using a Hamilton microsyringe. Measurements at each concentration were repeated three times at intervals of 60 s until the surface tension data was constant with time. The measured surface tension values were plotted as a function of the decadic logarithm of surfactant concentration. Prior to the measurements, the surface tension of the double distilled water was found to be 72.3 m N/m. The ring was flamed clean before the next measurement.

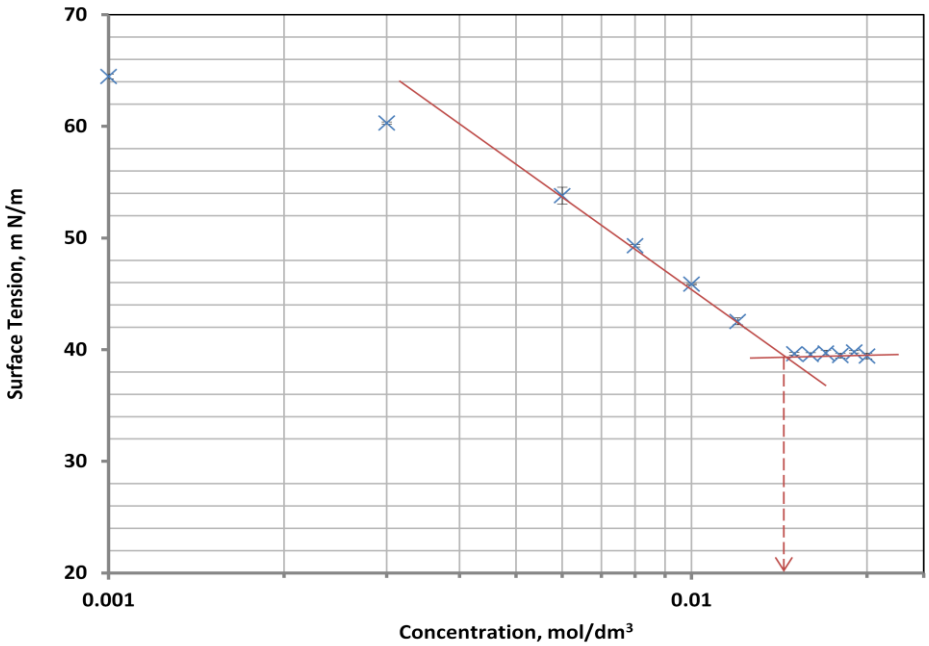


Figure 2.4 : Example of determination of CMC values for C_{12}TAB surfactants.

2.4.1.3 Theoretical CMC Values

From the surface tension measurement, a series of analysis were done to understand the phase behaviour of surfactants at liquid interface. There are different types of approach used to study the phase behaviour of a surfactant system. This depends on the suitability of the surfactant's character to match the studied model. For our binary mixed surfactants system, ideal critical micelle concentration of the mixtures (CMC*) were calculated using the Clint's equation (Rosen, 1989) as below:

$$\frac{1}{\mathrm{CMC}^*} = \frac{\alpha_1}{\mathrm{CMC}_1} + \frac{\alpha_2}{\mathrm{CMC}_2} \qquad (1)$$

where α_1 denotes mole fraction of surfactant 1 (anionic) and α_2 denotes mole fraction of surfactant 2 (cationic), and CMC_1 and CMC_2 are the CMC values of single surfactants. In spite of its inherent limitations, the Clint's equation is very useful for comparison between the ideal and nonideal situations.

2.4.1.4 Interaction Parameter of Surfactant Molecules

According to Rubingh's regular solution and phase separation model of micellization approximation (Rosen, 1989), the deviation of CMC and CMC* can be studied. The excess Gibbs energy of mixing is assumed to consist of only an enthalpy term, while there are no excess entropy contributions. This approximation may have limitations to the interpretation of CMC data, but regular solution theory continues to be widely used. In this model, calculation of the interaction parameter in a mixed micelle, β_{mic} can be achieved by using the following equation

$$\beta_{mic} = \frac{\ln\left(\frac{\alpha CMC_{mix}}{X_{mic} CMC_1}\right)}{(1-X_{mic})^2} \quad (2)$$

where CMC_{mix} is the CMC of surfactant mixtures, CMC_1 is the CMC of surfactant 1 and X_{mic} is the mole fraction of surfactant 1 in the mixed micelle. X_{mic} can be determined by Equation 3.

$$\frac{(X_{mic})^2 \ln\left(\alpha\frac{CMC_{mix}}{X_{mic} CMC_1}\right)}{(1-X_{mic})^2 \ln\left(\frac{(1-\alpha)CMC_{mix}}{(1-X_{mic})CMC_2}\right)} = 1 \quad (3)$$

Meanwhile, the interaction parameter for mixed monolayer formation at the aqueous liquid/air interface, β_{mon} can be calculated as below:

$$\beta_{mon} = \frac{\ln\left(\frac{\alpha C_{mix}}{X_{mon} C_1}\right)}{(1-X_{mon})^2} \quad (4)$$

where C_{mix}, C_1 and C_2 are the concentrations of mixed surfactant, surfactant 1 and surfactant 2, respectively, required to reduce the water surface tension by 30 mNm^{-1} (Figure 2.5).

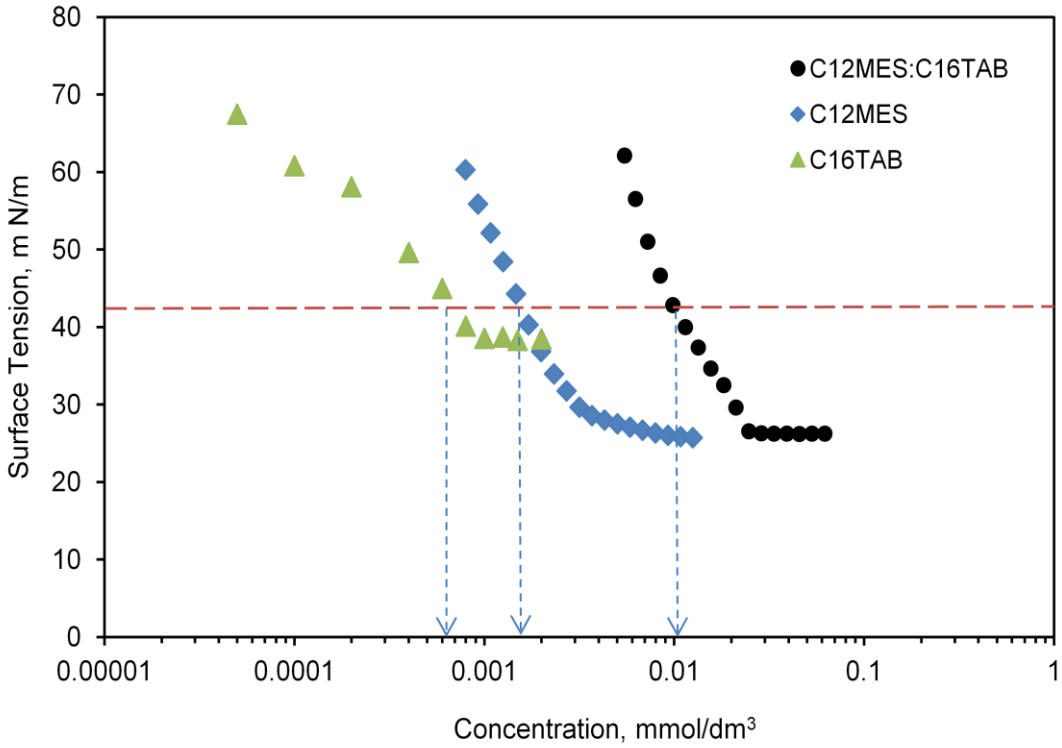


Figure 2.5 : Example of determination of C_{mix}, C_1 and C_2 for mixed surfactant, surfactant 1 and surfactant 2 which reduced the water surface tension by 30 mNm^{-1}.

X_{mon} is the mole fraction of surfactant 1 in the mixed monolayer which can determined from the following equation:

$$\frac{(X_{mon})^2 \ln\left(\alpha \frac{C_{mix}}{X_{mon} C_1}\right)}{(1-X_{mon})^2 \ln\left(\frac{(1-\alpha) C_{mix}}{(1-X_{mon}) C_2}\right)} = 1 \qquad (5)$$

2.4.1.5 Free Energy of Mixing

In addition, β_{mic} can also be related to the activity coefficients (g_1 and g_2) of the surfactants within the micelle by using the following equations:

$$g_1 = \exp\{\beta_{mic}(1 - X_{mic})^2\} \quad (6)$$

$$g_2 = \exp\{\beta_{mic}X_{mic}^2\} \quad (7)$$

Consequently, activity coefficients can be further used to calculate the excess Gibbs free energy of mixing, ΔG°_{ex} using Eq. 8

$$\Delta G^{\circ}_{ex} = RT[X_1 \ln g_1 + (1 - X_1)\ln g_2] \quad (8)$$

in which R and T are gas constant and absolute temperature, respectively.

2.4.1.6 Properties at the Air/Water Interface

Interfacial properties of the surfactant molecules at the aqueous liquid/air interface can be studied by measuring the surface excess concentration (Γ_{max}, mol/m^2). Normally, the concentration of surfactant is denser on the surface monolayer as compared to the bulk system. Γ_{max} can be determined from the slope of surface tension versus ln (concentration) curve. The values of the slopes (Figure 2.6) are then applied to the following equation:

$$\Gamma_{max} = -\left(\frac{1}{2.3\ nRT}\right)\left(\frac{d\gamma}{d\ln C}\right) \quad (9)$$

where C is the concentration, R is the gas constant (8.31 J mol^{-1} K^{-1}) and T is the absolute temperature. The n prefactor represents the number of species at the interface, the concentration of which changes with the surfactant concentration. The value depends on the degree of dissociation of ionic surfactants, which we do not know exactly. For a relative comparison within a series of compound, complete dissociation was assumed for all surfactant systems. From the Γ_{max} values, we can calculate the minimum area per molecule (A_{min}) of the surfactants at the air/water interface. A_{min} can be evaluated in the unit of nm^2 by using Eq. 10:

$$A_{min} = \frac{10^{18}}{\Gamma_{max} N_A} \tag{10}$$

in which N_A is Avagadro's number.

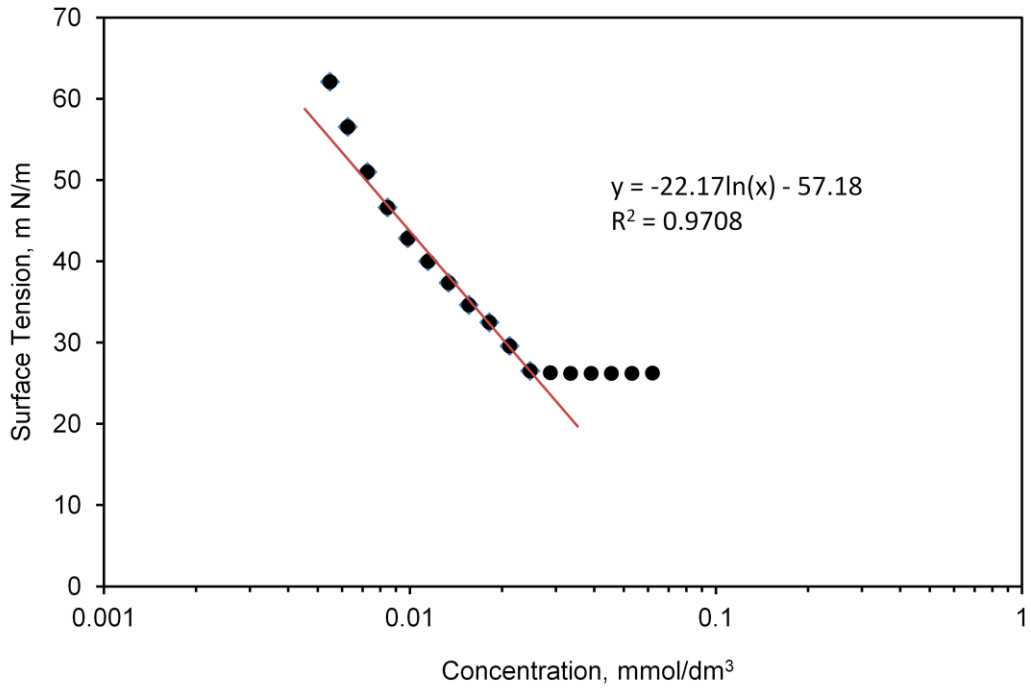


Figure 2.6 : Example of determination of slope values from the surface tension plots of C_{12}MES/C_{16}TAB mixtures at the mixing ratio of 0.80:0.20.

2.4.2 Catanionic Surfactants

2.4.2.1 Preparation of Catanionic Solution

Stock solution of catanionic surfactants were prepared by weighing approximately 2-20 mg of C_mMES.C_nTAB solids into 100 mL of distilled water. The catanionic stock solutions were then vortexed for 5 minutes at 2500 rpm using Stuart Vortex Mixer – SA8, USA. The catanionic solutions were kept for 24 hours in water bath at 30˚C before performing surface tension measurements.

Concentration of the catanionic stock solution prepared was different for each system depending on the approximate CMC values obtained from test run. C_{12}MES.C_{12}TAB and C_{12}MES.C_{14}TAB catanionic systems were prepared at a same concentration which is 0.6 mM. C_{12}MES.C_{16}TAB and C_{12}MES.C_{18}TAB catanionic systems were prepared at 0.05 mM. All C_{14}MES.C_nTAB catanionic systems were prepared at 0.02 mM. Similarly, C_{16}MES.C_nTAB catanionic solutions were also prepared at 0.02 mM.

2.4.2.2 Surface Tension Measurement

The critical micelle concentration (CMC) for catanionic surfactants solutions were determined by surface tension (γ) measurement using a du Nouy ring tensiometer (KSV Sigma 70, Helsinki). Surface tension for all catanionic samples were measured at 30±0.1˚C. Concentration of surfactant was varied by dilution of stock surfactant solution with distilled water using a Hamilton microsyringe. Each concentration was measured three times at intervals of 60 s until the surface tension data were constant with time. The measured surface tension values were plotted as a function of the decadic logarithm of surfactant concentration. Prior to the measurements, the surface tension of the double distilled water was found to be 72.3 m N/m. The ring was flamed

clean before the next measurement. All samples are measured three times and an average value is taken.

2.4.2.3 Properties at the Air/Water Interface

Aside from determination of CMC values, further analysis can be done to understand the surface activities of catanionic surfactants. One of the important factors that contribute to the surface performance of a surfactant is the ability of surfactant molecules to adsorb at the liquid/air interface. The higher the concentration of surfactants, the greater is the surface activity. The direct determination of the amount of surfactant molecules adsorbed per unit area of liquid/air interface can be done by measuring the surface excess concentration ($\Gamma_{max'}$, mol/m^2). The values of the slopes of surface tension versus ln (concentration) curve are applied to the following equation derived from the Gibbs adsorption equation:

$$\Gamma_{max'} = -\left(\frac{1}{4.606\ RT}\right)\left(\frac{d\gamma}{dlnC}\right) \quad (11)$$

where C is the concentration, R is the gas constant (8.31 J mol^{-1} K^{-1}) and T is the absolute temperature. For a relative comparison within a series of compound, complete dissociation was assumed for all surfactant systems. From the $\Gamma_{max'}$ values, we can calculate the minimum area per molecule ($A_{min'}$) of the surfactants at the air/water interface. $A_{min'}$ can be evaluated in the unit of nm^2 by using Eq. 12:

$$A_{min'} = \frac{10^{18}}{\Gamma_{max} N_A} \quad (12)$$

in which N_A is Avagadro's number.

2.5 Anti Bacterial Properties

2.5.1 Bacterial Culture

S. aureus (ATCC 6538) was used as an example for Gram-positive bacteria while; *E. coli* (ATCC 8739) and *P. aeruginosa* (ATCC 15442) were used to represent Gram-negative bacteria in our study. These bacteria were selected due to their common occurrence in topical pharmaceutical products. Although *P. aeruginosa* (PA) is not facultative anaerobic bacteria like *S. aureus* (SA) and *E. coli* (EC), but it is well adapted to proliferate in conditions of partial or total oxygen depletion. Therefore, all three types of bacteria were cultured under the same method and condition. Trypticase Soy Agar (TSA, Fluka) was used as the nutritive media. All glasswares, apparatus and distilled water were autoclaved before use.

Firstly, a colony of a particular bacteria was carefully picked up using an inoculation loop (sterilized by flame from a Bunsen burner). The loop containing the bacteria was then streaked at the top end of a new agar plate, moving in a zig zag horizontal pattern until 1/3 of the plate is covered. The loop was sterilized again before the next streaking process. After that, the plate was rotated 60 ° and the bacteria from the end of the first streak were spread into a second area using the same motion. The same procedure was repeated for third streaking area (Figure 2.7). The plate was then sealed with parafilm, inverted and incubated at 37˚C for 24 hours.

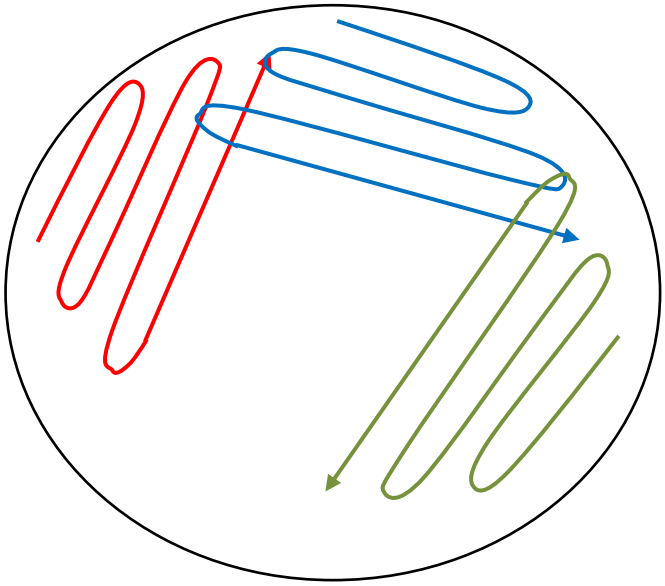


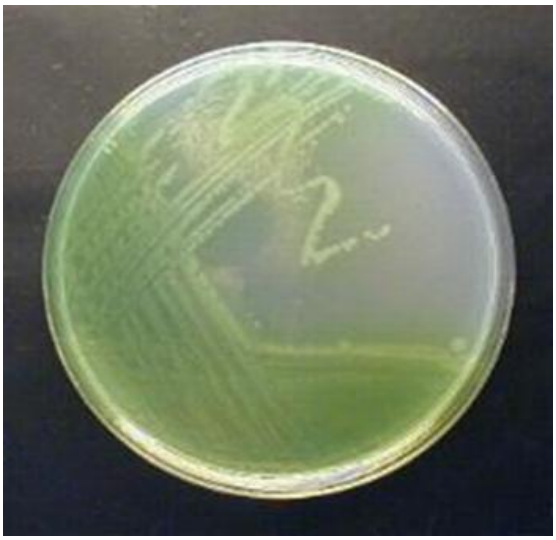
Figure 2.7 : Direction of streaking bacteria on a culture plate. Red line indicates first streak series, blue for second streak series and green for third streak series.

After 24 hours, bacterial cell growth was observed to confirm that pure cultures were formed. From our observation, SA forms circular, pinhead colonies which are convex with entire margins. The colonies are mainly golden brown in colour. EC will form shiny, mucoid colonies which have entire margins and are slightly raised. Meanwhile, PA forms mucoid colonies with umbonate elevation and produce a diffusable green pigment. Any irregularities to the growth of the bacteria will void its usage for further testing (Figure 2.8).

After identifying the pure culture, one colony was picked using a sterilized inoculation loop and dipped into a test tube containing approximately 5 mL of water. The loop was gently tapped to shake off the bacteria. Then, the test tube was vortexed at 1000 rpm for 2 minutes to disperse the bacteria uniformly. Final suspension was adjusted to 0.5 (Mc Farland scale), which is also equivalent to 1.5×10^8 cfu/mL.

(a)

(b)



(c)

Figure 2.8 : Examples of bacteria cultures (a) SA, (b) EC and (c) PA after 24 hours of incubation.

2.5.2 Preparation of Anionic/Cationic Mixed Surfactant Solution

Stock solution for both single surfactants C_mMES and C_nTAB were prepared by dissolving approximately 0.8-1.0 g of solid samples into 100 mL of autoclaved, distilled water. The final concentration of the stock solution will come up to 25 mM. Mixed surfactants were then prepared by mixing the stock solution of C_mMES and C_nTAB

surfactants according to the desired molar ratio (C_mMES/C_nTAB) i.e; 25:75, 50:50 and 75:25, where m= 12, 14, 16 and n= 12, 14, 16, 18 carbon number in the hydrophobic chain. C_mMES was added drop wise into solution of C_nTAB and the mixtures were vortexed for 5 minutes at 2500 rpm. The stock solutions were sterilized by filtering through cellulose acetate filters (0.2µm pore size; Millipore) in a laminar flow cabinet. The mixed solutions were kept for 24 hours in water bath at 35˚C before performing bacteria inhibition test.

2.5.3 Preparation of Catanionic Surfactant Solution

Stock solution of catanionic surfactants were prepared by weighing approximately 2-20 mg of C_mMES.C_nTAB solids into 100 mL of autoclaved, distilled water. The final concentration of the stock solution will be 12.5 mM. The catanionic stock solutions were then vortexed for 5 minutes at 2500 rpm. The stock solutions were sterilized by filtration through cellulose acetate filters (0.2µm pore size; Millipore) under laminar flow at room temperature. Before performing bacteria inhibition test, catanionic solutions were kept for 24 hours in water bath at 35˚C.

2.5.4 Minimum Inhibition Concentration (MIC) Assay

Minimum inhibitory concentration (MIC) assay is an *in vitro* technique used to determine the lowest concentration of an antibacterial agent needed to kill bacteria. This assay is typically performed on planktonic (free floating) bacterial cells. For the present studies, dilution method (Andrews, 2001; Sütterlin *et al.*, 2008) was selected to determine MIC values for all single surfactants, anionic/cationic mixtures and catanionic surfactants. Several steps were modified to suit the testing of the selected antibacterial agents.

A series of dilutions were made to the stock solution of the surfactants prepared in section 2.5.2 and 2.5.3. Interaction between surfactants and monoculture strain of SA, EC and PA was induced by adding 100 µL bacteria suspensions prepared in section 2.5.1 into 9.9 mL of surfactant solutions. The final concentrations of surfactant solutions with bacteria were 12.5, 1.25, 0.125, 0.0125 and 0.00125mM. The mixtures of surfactants and bacteria were then vortexed at 1000 rpm for 2 minutes. A positive control without any surfactant added was also prepared as reference. Test tubes containing the test samples were then incubated at 37˚C for 1 hour.

After that, 100 µL of the mixtures of surfactant and bacteria were spread on agar plate and incubated for 24 hours at 37˚C. The effect on bacteria growth was determined by visual counting and MIC values were determined using scientific research software as shown in Figure 2.9. IC_{90} is defined as the lowest concentration of surfactant at which there is a decrease of 90% in bacteria colonies as compared to the positive control (Sütterlin *et al.*, 2008)

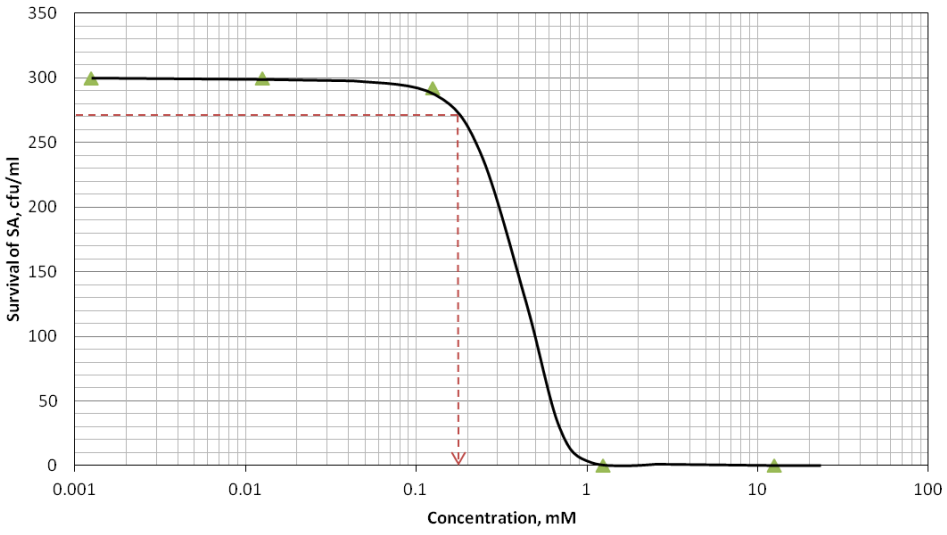


Figure 2.9 : Example of determination of IC_{90} values for C_{16}MES on S. *Aureus*.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Formation of Catanionic Surfactants

As mentioned in Section 2.2.1, catanionic surfactants are obtained from the equimolar mixing of cationic and anionic surfactants with removal of the low molecular weight salt. In this novel surfactant system, the polar head of each surfactant is actually the counterion of the oppositely charged surfactants. Due to this unique characteristic, catanionic surfactants behave as a single net neutral molecule with a wide array of morphologies in water (Khan and Marques, 1997; Tondre and Caillet, 2001; Marques *et al*., 2003). To date, there are three ways of preparing catanionic surfactants which are the precipitation method, solvent extraction method and ion exchange method.

In the present work, the precipitation method was chosen to prepare C_mMES.C_nTAB catanionic surfactants. This is simply because the method is straightforward, leads to less complications and suits the C_mMES.C_nTAB catanionic surfactant systems. However, C_mMES.C_nTAB catanionic surfactants have a tendency to form small crystals and lamellar phases which are difficult to be filtered out (Silva and Marques, 2005; Li *et al*., 2004). A few measures were taken to overcome this problem.

Firstly, it's important to make sure that precipitates of C_mMES.C_nTAB catanionic surfactants were formed instead of other form of aggregations (*i.e.*; lamellar phase, micelles, vesicles *etc.*). As mentioned earlier, factors affecting the phase behaviour of a surfactant system include total concentration, mixing ratios and temperature (Guo & Szoka, 2002; Zhao *et al.*, 2012; Matos *et al.*, 2013; Li *et al.*, 2013). Since the mixing

ratio of a catanionic surfactant is fixed at 1:1 molar ratio, we can only play with the total concentration and temperature parameters.

All C_mMES.C_nTAB catanionic surfactants were prepared in a series of concentrations ranging from 0.5 – 2.5 mM and their aggregation behaviours were observed for 48 hours. An example of observation for C_{12}MES.C_{12}TAB catanionic surfactant is showed in Figure 3.1. It can be seen that C_{12}MES.C_{12}TAB catanionic surfactant formed a precipitate at the concentration of 1.25 mM. A concentration of 2.5 mM was too high as a lamellar phase was formed, meanwhile, a concentration of 0.5 mM was too low as only micelles and vesicles were formed. Optimum concentrations for the formation of all C_mMES.C_nTAB catanionic precipitates are listed in Table 3.1.

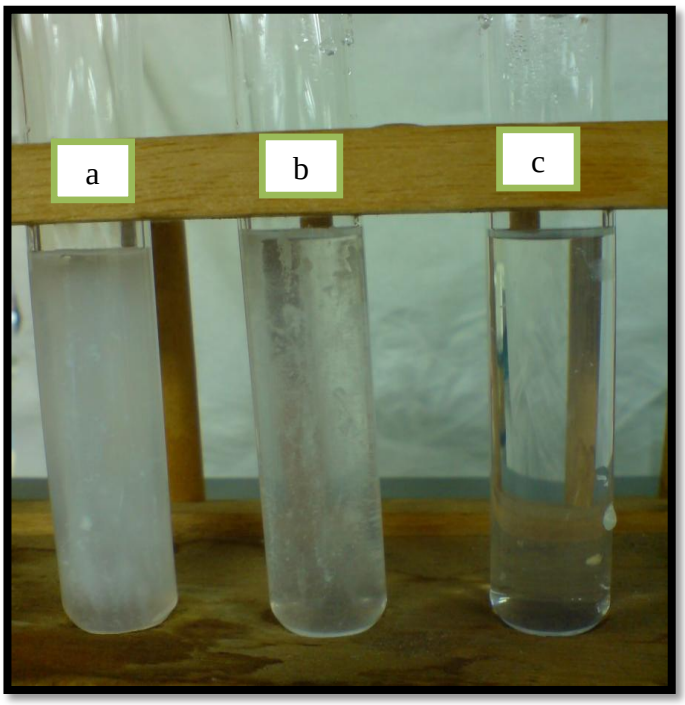


Figure 3.1 : Aggregation behaviour of C_{12}MES.C_{12}TAB catanionic surfactant at (a) 2.5 mM, (b) 1.25 mM and (c) 0.5 mM.

Table 3.1 : Optimum concentration where C_mMES.C_nTAB catanionic surfactants form precipitates and its average yield.

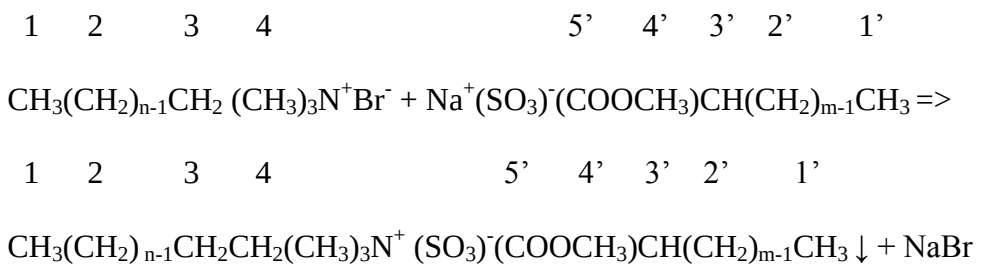
Catanionic System	Concentration, mM	% Yield
C_{12}MES.C_{12}TAB	1.25	76.5
C_{12}MES.C_{14}TAB	2.50	84.5
C_{12}MES.C_{16}TAB	2.50	95.0
C_{12}MES.C_{18}TAB	1.25	72.5
C_{14}MES.C_{12}TAB	1.25	72.5
C_{14}MES.C_{14}TAB	1.25	86.5
C_{14}MES.C_{16}TAB	1.25	85.0
C_{14}MES.C_{18}TAB	2.50	95.0
C_{16}MES.C_{12}TAB	1.25	76.0
C_{16}MES.C_{14}TAB	1.25	88.0
C_{16}MES.C_{16}TAB	1.25	84.5
C_{16}MES.C_{18}TAB	0.50	77.5

From the trials, it was found that some C_mMES.C_nTAB catanionic surfactant systems were easier to prepare as compared to others in terms precipitate formation. For instance, C_{12}MES.C_{16}TAB and C_{14}MES.C_{18}TAB catanionic surfactants are easiest to prepare and have high yield. This is due to the alkyl chain length of anionic and cationic surfactants complimenting each other and favours the formation of precipitates. Meanwhile, precipitates of C_{12}MES.C_{18}TAB, C_{14}MES.C_{12}TAB and C_{16}MES.C_{12}TAB systems were quite difficult to form due to the mismatch of the alkyl chain length (Fernandes *et al.*, 2010; Jiao *et al*., 2013).

In addition, we have also adopted a cold filtration system to aid the filtration process which runs at 5℃. A lower temperature promotes the formation of precipitates as the surfactant molecules can pack closer to each other. For C_{12}MES.C_{18}TAB, C_{14}MES.C_{12}TAB and C_{16}MES.C_{12}TAB systems, recrystallization with acetone was needed. Such measures help in the formation of larger crystals and leads to easier filtration.

The ion exchange method would not be recommended because acidifying C_mMES will interfere with its stability and may lead to the formation of diacid alkyl ester sulfonate (H_2C_mES). Similarly, H_2C_mES can also pair with C_nTAB via electrostatic interactions to form H_2C_mES.C_nTAB catanionic surfactants which are hard to distinguish from the desired C_mMES.C_nTAB system. On the other hand, the solvent extraction method is not suitable for the various alkyl chain lengths of C_mMES.C_nTAB catanionic systems. For certain alkyl chain length, C_mMES.C_nTAB system tends to assemble at the interface of the two immiscible media. This causes difficulty in separating high purity C_mMES.C_nTAB catanionic system.

The spontaneous formation of the catanionic surfactant can be represented as follows:



3.1.1 ^{1}H NMR Analysis

Detailed ^{1}H NMR spectra recorded for various single C_nTAB surfactants at 298K are given in Appendix 1-4, and for the catanionic surfactants are presented in Appendix 5-16. Here ^{1}H NMR spectra of $C_{18}TAB$ single surfactant and $C_{12}MES.C_{18}TAB$ catanionic surfactant recorded at 400 MHz are shown in Figure 3.2 and Figure 3.3 respectively. Characteristic ^{1}H NMR chemical shifts of $C_{12}MES.C_{18}TAB$ catanionic are indicated in the ^{1}H NMR spectra. The terminal 1 and 1'-CH_3 protons of the catanionic surfactantshowed a triplet at ~0.87 ppm. The resonance of 2, 2'-CH_2– protons of the long chain from both $C_{12}MES$ and $C_{18}TAB$ showed a superimposed single absorption at 1.25 ppm with a broad downfield side. The methylene protons (–CH_2–) attached to the ammonium group in $C_{18}TAB$ exhibited triplet at 3.39 ppm. The 4–CH_3– next to the ammonium group in $C_{18}TAB$ formed a singlet at 3.27 ppm while 4'–CH_3– from the ester group in $C_{12}MES$ formed a singlet at 3.73 ppm. The 4 –CH_3– and 3 –CH_2– of ammonium head group of $C_{12}MES.C_{18}TAB$ catanionic surfactants produced an shift of ~0.21 and 0.19 ppm with respect to the pure cationic surfactant ($C_{18}TAB$). This is due to the electrostatic field in the catanionic surfactants was changed when the bromide ion (Br-) of $C_{18}TAB$ was replaced by sulfonate ion (SO_3^-) in $C_{12}MES$ head group (Tomašić *et al.*, 1997; Zhao and Fung, 1993). Similar results from the works of Maiti and co-workers (2010) were reported for CP^+DS^- (1:1 coacervate of cetylpyridinium chloride (CPC) and sodium dodecylsulfate (SDS).

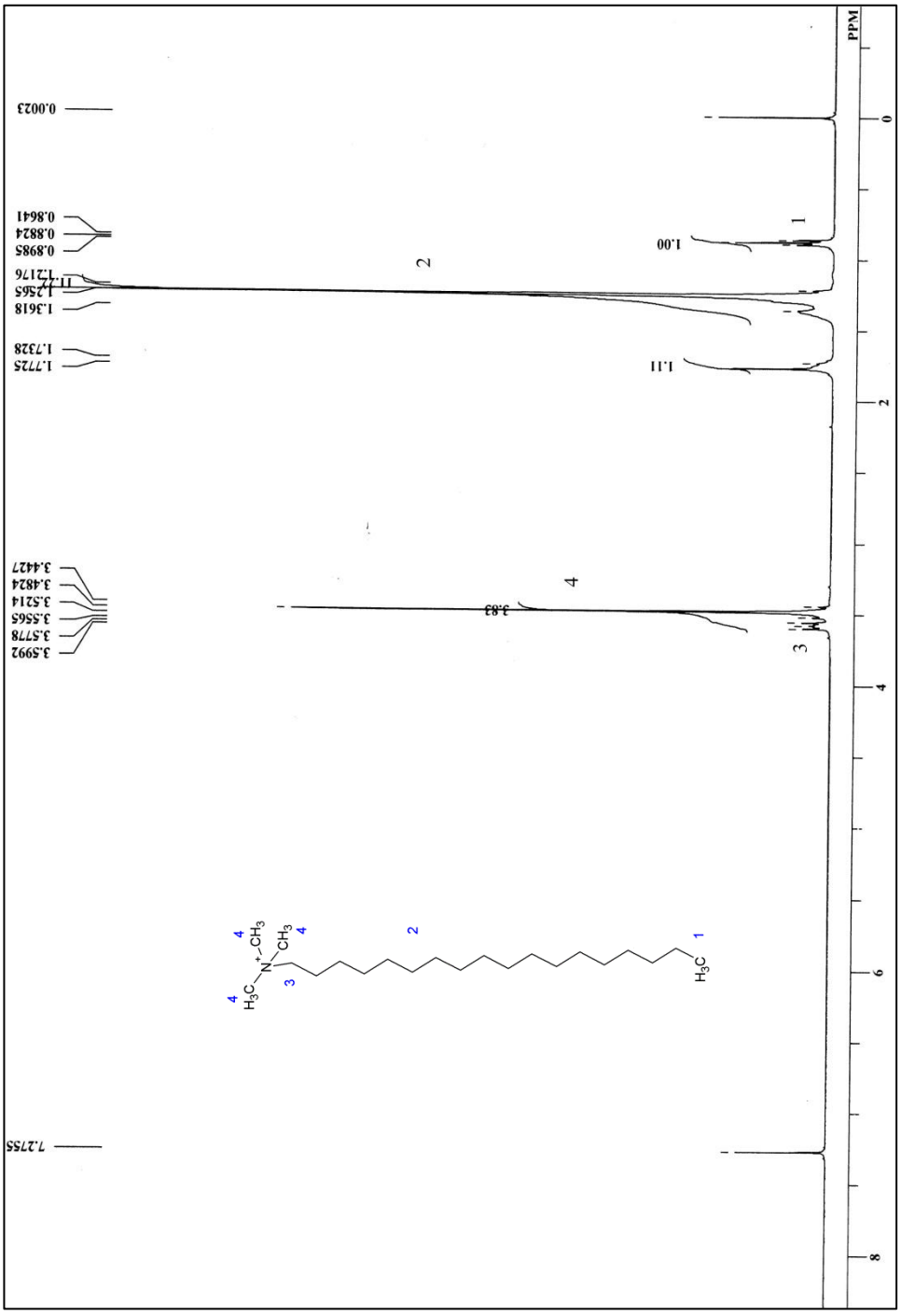


Figure 3.2 : ^{1}H NMR spectrum of C_{18}TAB single surfactant recorded at 400 MHz.

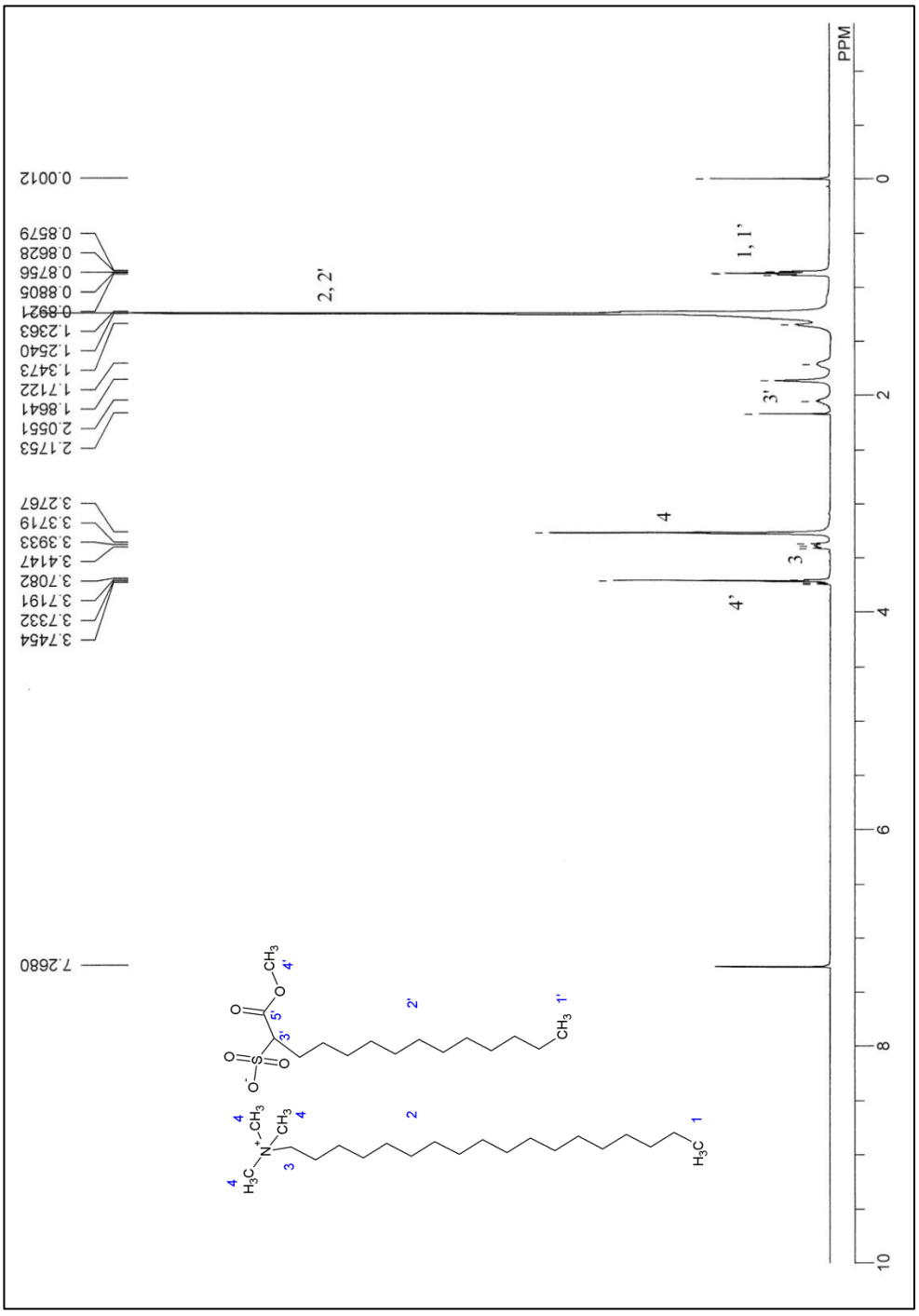


Figure 3.3 : ^{1}H NMR spectrum of C_{12}MES.C_{18}TAB catanionic surfactant recorded at 400 MHz.

3.1.2 ^{13}C NMR Analysis

The ^{13}C NMR chemical shifts measured for C_{14}TAB single surfactant and C_{14}MES.C_{14}TAB catanionic surfactant at 125 MHz are shown in Figure 3.4 and Figure 3.5 respectively. The ^{13}C chemical shift values were referred to the center peak of $CDCl_3$ which has a value of 77.18 ppm. The 4 -CH_3- and 3 –CH_2- peaks of the catanionic systems, C_{14}MES.C_{14}TAB were slightly shifted (approximately 0.3 ppm) as compared to single surfactants, C_{14}TAB. Whereas, peaks for 1, 1' -CH_3- and, 2, 2'–CH_2- remain unchanged. A similar pattern was also observed from ^{1}H NMR analysis. This further confirmed that there is a change in the electrostatic field (Zhao and Fung, 1993). at the surfactant head group region. The rest of the ^{13}C NMR spectra for the pure surfactants and the catanionic surfactants are shown in Appendix 17-32.

The following were ^{13}C NMR results of C_nTAB and catanionic (C_mMES.C_nTAB) at 298K.

C_nTAB (100 MHz, $CDCl_3$) δ/ppm: 14.13 (CH_3, 1), 22-32 (CH_2, 2) , 53.39 (CH_3, 4), 67.10 (CH_2, 3).

C_mMES.C_nTAB (100 MHz, $CDCl_3$) δ /ppm: 14.17 (CH_3, 1, 1'), 22-32 (CH_2, 2, 2'), 53.09 (CH_3, 4), 66.82 (CH_2, 3), 170.50 (CO, 5').

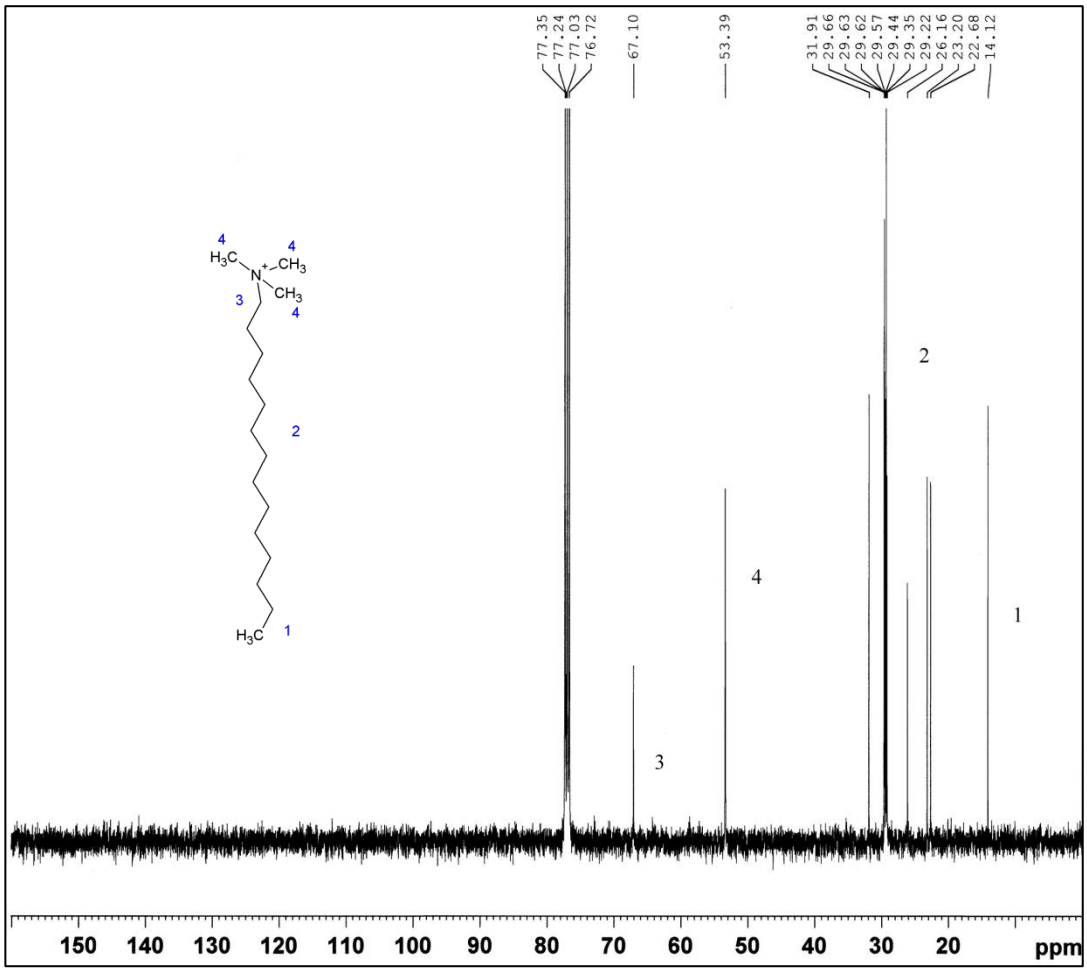


Figure 3.4 : ^{13}C NMR spectrum of C_{14}TAB single surfactant recorded at 125 MHz.

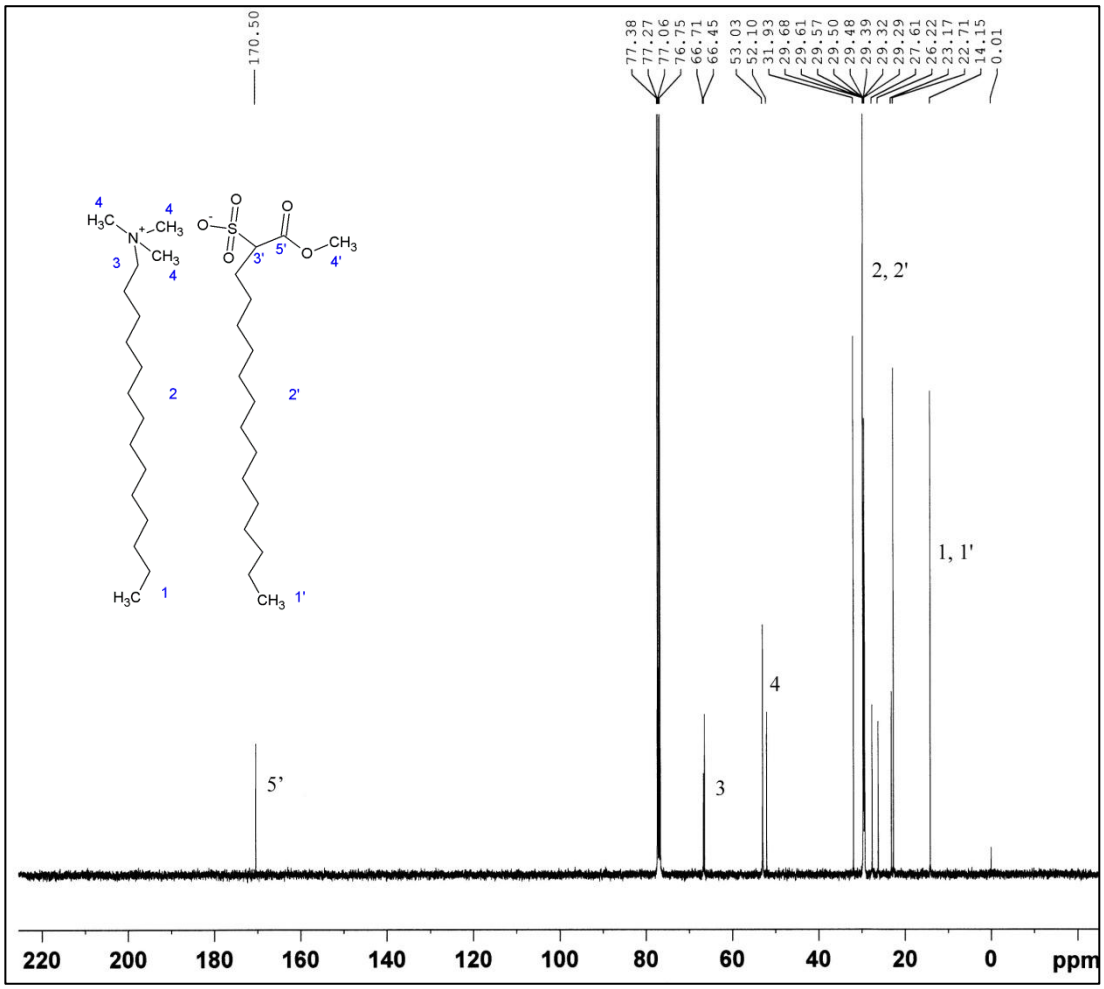


Figure 3.5 : ^{13}C NMR spectrum of $C_{14}MES.C_{14}TAB$ catanionic surfactant recorded at 125 MHz.

3.1.3 FTIR Analysis

The peak positions of FTIR spectra for C_nTAB single surfactants and $C_mMES.C_nTAB$ catanionic surfactants at 298K are identified in Table 3.2. $C_{14}MES$ anionic surfactant, $C_{18}TAB$ cationic surfactant and $C_{14}MES.C_{18}TAB$ catanionic surfactant were chosen as example for FTIR analysis discussion. The FTIR spectra of the three selected surfactants are shown in Figure 3.6, 3.7 and 3.8 respectively. The symmetric and asymmetric methyl and methylene (C–H) stretching frequencies of the catanionic were more or less at the same position as in the parent surfactants. Changes in the peak positions were only observed in the head groups region. The symmetric (S–O) stretching frequencies were shifted to 1340 cm^{-1}, approximately 9 cm^{-1} lower as compared to the pure $C_{14}MES$. Meanwhile, asymmetric ($H_3C–N^+$) stretching frequencies were shifted from 962 cm^{-1} to 974 cm^{-1}, which is 12 cm^{-1} higher than $C_{18}TAB$. Considerable changes observed in the regions (1750–1160cm^{-1}) and (962–718cm^{-1}) arose due to size and charge density differences of Na^+ and alkyltrimethylammonium ion. The results suggest ionic bond formation in the coacervate. According to Tomašić *et al*., (1997) the symmetric (S–O) and asymmetric ($H^3C–N^+$) stretching frequencies of $C_{16}TA^+DS^-$ were shifted to 23cm^{-1} lower and 50cm^{-1} higher frequencies, with respect to pure SDS and $C_{16}TAB$. FTIR spectra of single C_nTAB and $C_mMES.C_nTAB$ are presented in Appendix 33-48.

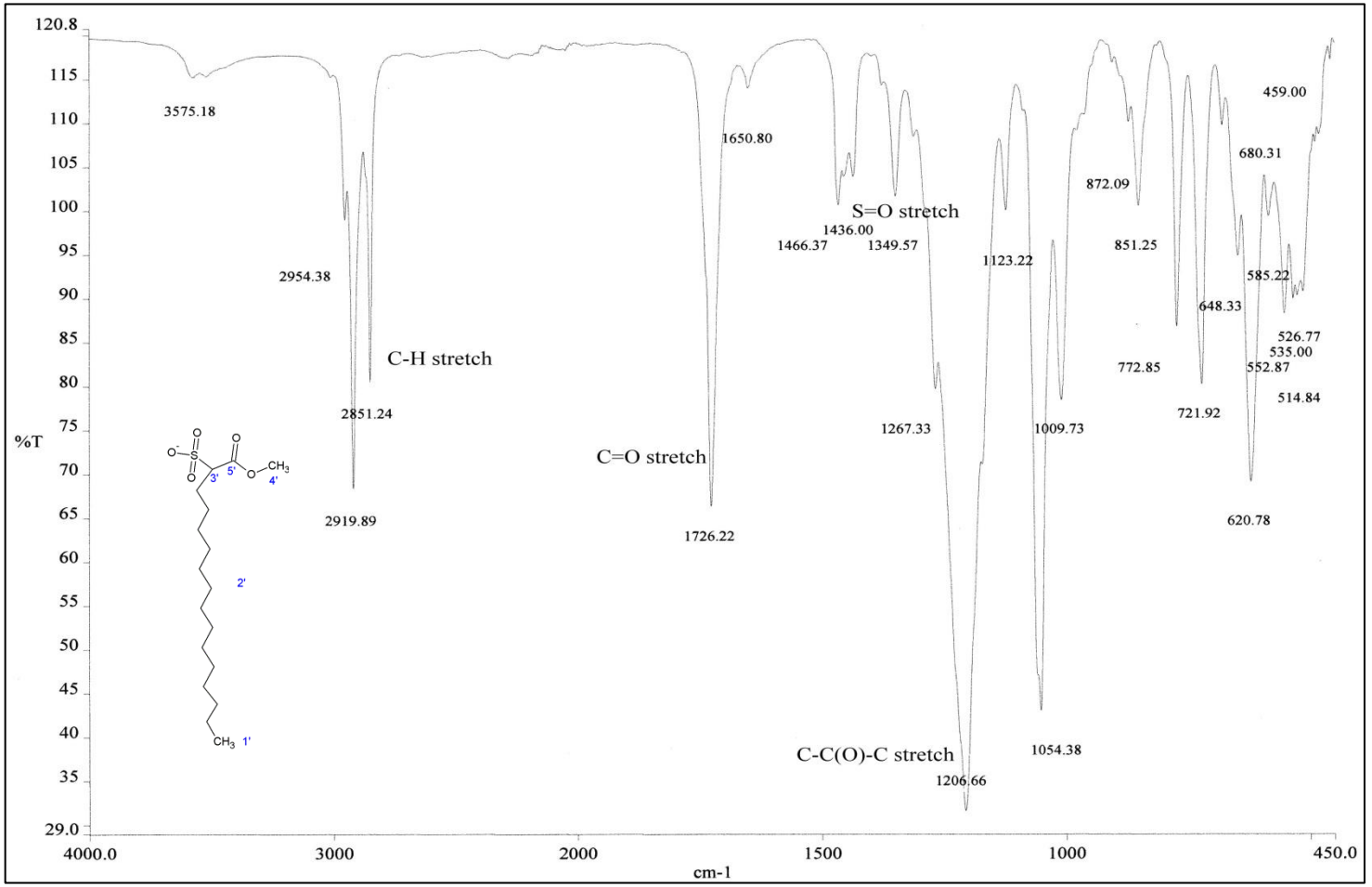


Figure 3.6 : FTIR spectrum of C_{14}MES anionic surfactant recorded at 298K

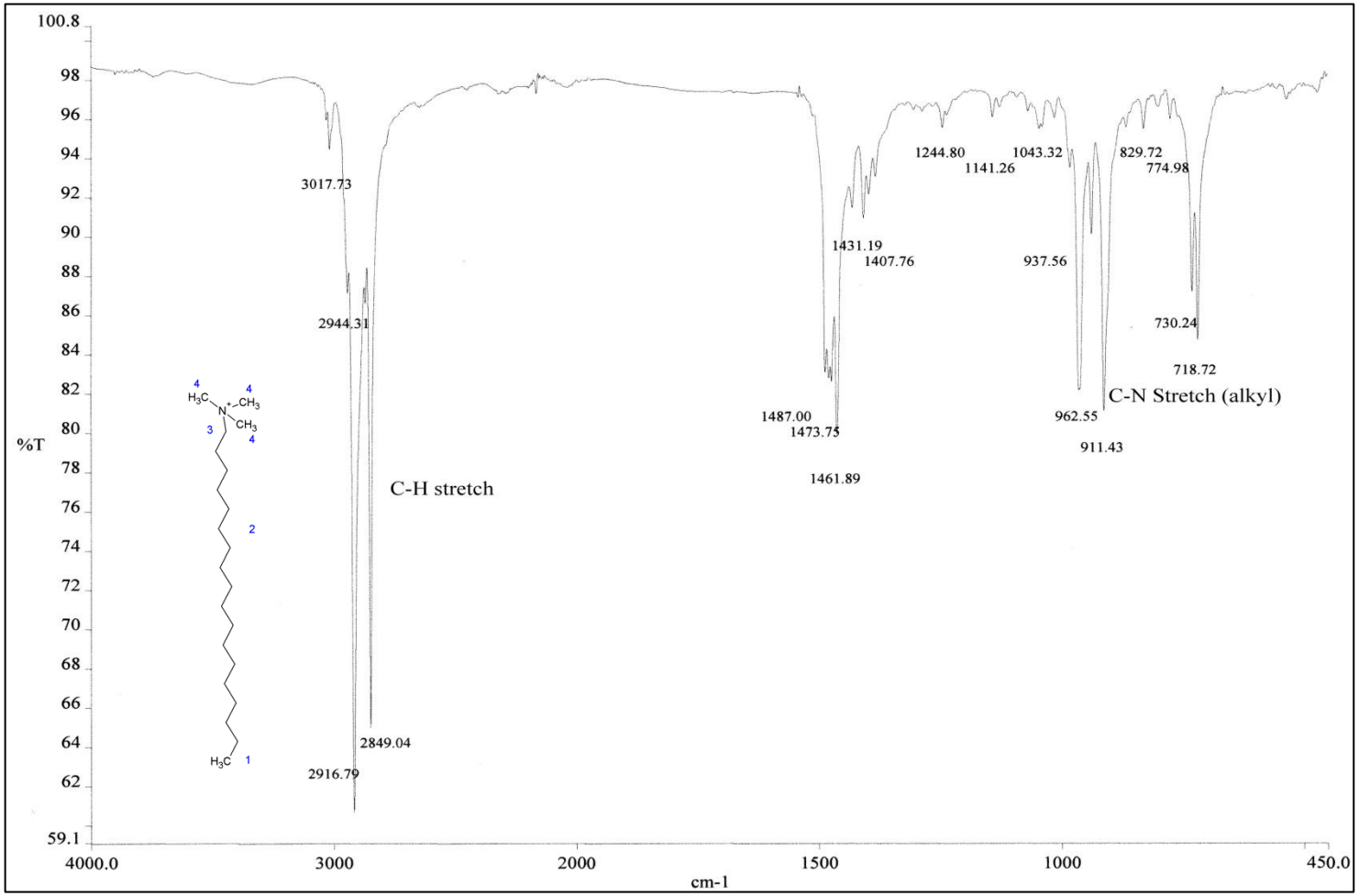


Figure 3.7 : FTIR spectrum of C_{18}TAB cationic surfactant recorded at 298K

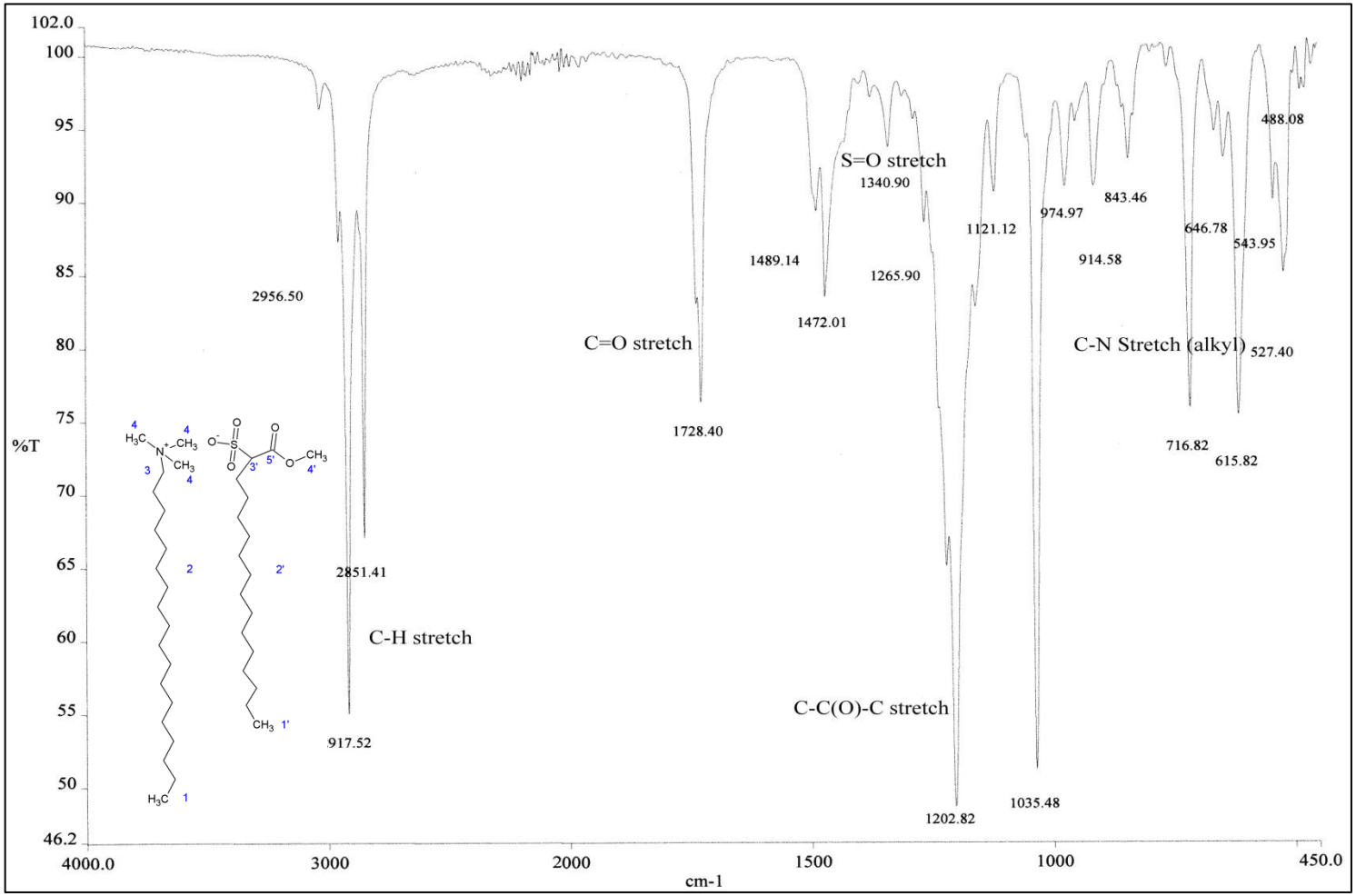
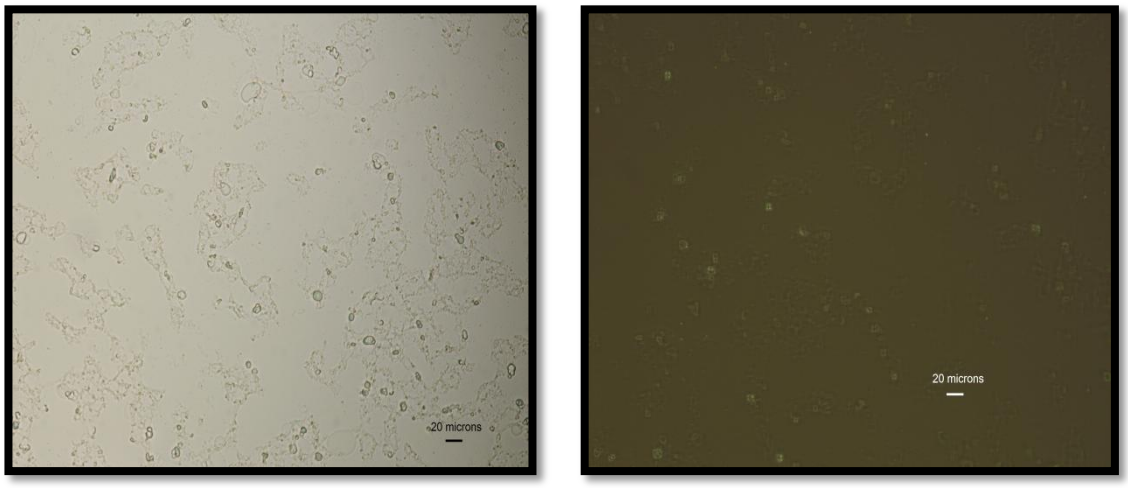


Figure 3.8 : FTIR spectrum of C_{14}MES.C_{18}TAB catanionic surfactant recorded at 298K.

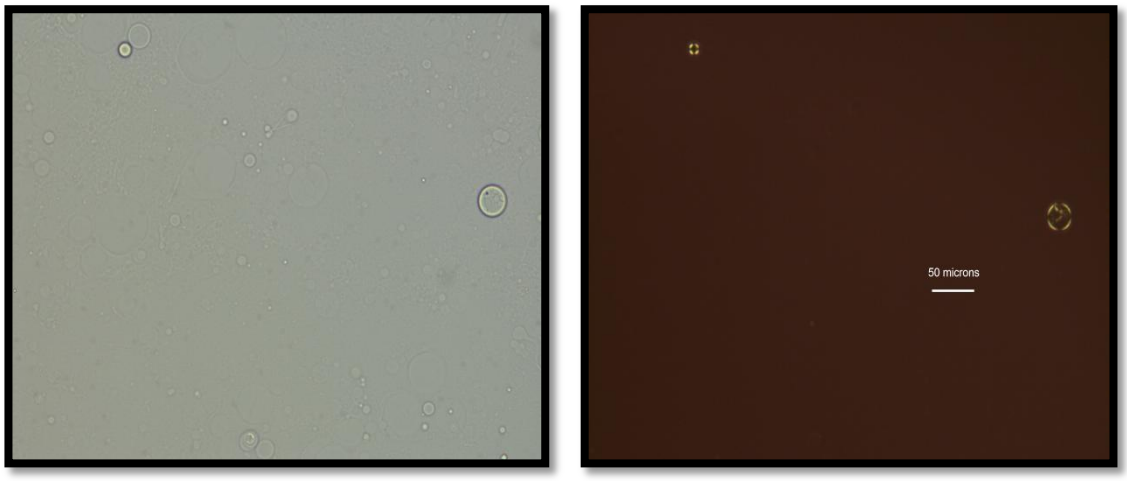
3.1.4 Polarized Light Microscopy Analysis

The aggregrate behaviour of catanionic surfactants was studied using optical polarizing microscope. All catanionic surfactants formed mesophases at low concentration (1.0 $mgmL^{-1}$) in water solutions at 25 ℃. Some of the micrographs are presented in Figure 3.9-3.11. Part (a) in the figure shows the sample under normal light, meanwhile part (b) shows the same sample under polarized light. Birefringent maltese crosses are clearly observable from the catanionic sample, indicating the formation of vesicles (Šegota *et al*., 2006; Blanzat *et al*., 1999; Marques *et al*., 2008). This spontaneous formation of vesicles is highly sought after in drug delivery application (Castagnos *et al*., 2010; Liu *et al*., 2013; Zhao *et al*., 2013)



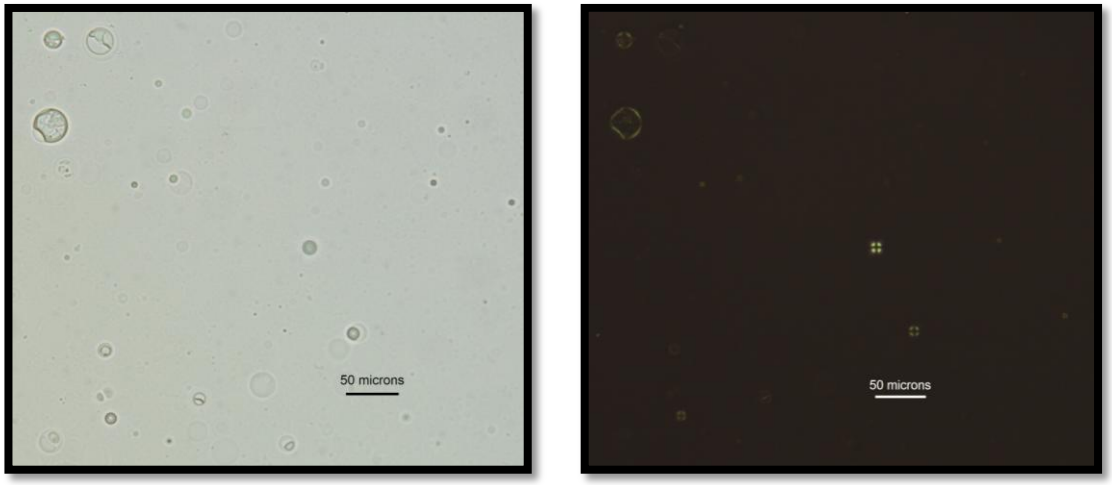
(a) (b)

Figure 3.9 : Optical micrographs of vesicles in C_{12}MES.C_{12}TAB catanionic systems at 200X magnification under (a) normal light and (b) polarized light.



(a) (b)

Figure 3.10 : Optical micrographs of vesicles in C_{14}MES.C_{12}TAB catanionic systems at 200X magnification under (a) normal light and (b) polarized light.



(a) (b)

Figure 3.11 : Optical micrographs of vesicles in C_{16}MES.C_{12}TAB catanionic systems at 200X magnification under (a) normal light and (b) polarized light.

3.2 Physical Properties

3.2.1 Anionic/Cationic Mixtures

Similar to single surfactants, mixed surfactants also undergo a series of changes in physico-chemical properties throughout the micellization process (Kume *et al*., 2008; Haque *et al*., 1996). The interactions between the surfactant molecules determine the properties and morphology of the system. According to Rubingh's regular solution theory (Rosen, 1989), interactions between the different surfactant molecules in the mixed systems can be measured by calculating the β parameter values from the plots of surface tension, γ, versus the concentration of aqueous solutions of the individual surfactants and at least one mixture of them. Since the value of β is proportional to the free energy of mixing of the systems, a negative value of β indicates attractive interactions between two different surfactant molecules was stronger than the attractive interactions between similar surfactant molecules and vice versa. β value close to zero shows that there are little or no interactions between the surfactant molecules which is commonly referred as ideal mixing behaviour. From previous studies, it has been shown that the β value of anionic/cationic surfactant mixtures is highly negative (Graciaa *et al*., 1989; Zhou and Rosen, 2003). For instance, β values for mixtures of sodium dodecyl sulfate (SDS) and dodecyltrimethylammonium bromide (DTAB) were approximately -25 whereas for other non-anionic/cationic mixtures, β values were above -4 (Zhou and Rosen, 2003). This indicated that the attractive forces between the oppositely charged surfactant molecules were very strong (Dragčević *et al*., 1995; Olea and Gamboa, 2003; Góralcyzk and Kita, 1998; Cui and Canselier, 2000).

The β parameter for a mixed system can be calculated at the surface monolayer, β^{mon} and also in the micelles, β^{mic}. The value of β^{mon} is not the same as the value of β^{mic} for the same two surfactants under the same conditions. For most mixed systems, interaction in the mixed monolayer was greater than that in the mixed micelle (Sohrabi *et al*., 2008; Zhou and Rosen, 2003). Such behaviour is most sought after in the detergency industry as the strong synergistic interactions greatly enhance the surface properties of the surfactants (Sohrabi *et al*., 2008). However, there were cases where the interactions in the mixed micelle were stronger than the interactions in mixed monolayers because surfactant molecules can pack closer at the monolayer (Sohrabi *et al*., 2008). Usually, interactions between the surfactant molecules were dominated by the hydrophilic part of the surfactants (Rosen, 1989; Sohrabi *et al*., 2008; Zhou and Rosen, 2003).

In comparison with other anionic/cationic mixtures, we studied sodium alkyl methyl ester sulfonate (C_nMES), a relatively new anionic surfactant that have considerably good surface activities as compared to conventional anionic surfactants such as alkyl sulfate (AS) and linear alkylbenzene sulfonates (LAS) (Teruhisa, 1992; Stirton *et al*., 1961; Kapur *et al*., 1978). The MES exhibits better biodegradability than other anionic surfactants (Teruhisa, 1992; Stirton *et al*., 1961; Kapur *et al*., 1978). By mixing MES with cationic surfactant alkyltrimethylammonium bromide (C_nTAB) of different chain lengths, we obtained a more superior system in terms of surface activity as compared to single MES. CMC values were determined and compared between single systems and mixed systems. β values, maximum surface excess, (Γ_{max}) and minimum area per molecule (A_{min}) were calculated using Rubingh's theory from the surface tension data. The effect of varying hydrocarbon chain length of anionic surfactants and cationic surfactants on the mixed systems were also studied.

3.2.1.1 Critical Micelles Concentration (CMC)

In general, the CMC of a surfactant solution can be obtained from the break point of surface tension, γ against concentration, M plot. Israelachivili (1992) showed that the important factors that influence surfactants self-assembly in aqueous solutions are molecular structures of the surfactant, intermolecular interactions and conditions of the bulk solution (i.e. surfactant concentrations, mixtures of surfactants, electrolyte concentration, pH and temperature).

Surface tension measurements of single cationic surfactants (C_nTAB) and single anionic surfactants (C_mMES) of varying carbon chain length in diluted solution are shown in Figure 3.10 and 3.11 respectively. None of the single surfactants exhibit any minimum value in the surface tension versus concentration curve. CMC values of all single surfactants obtained from our experiments were comparable to prior reported values (Murphy and Taggart, 2002; Sohrabi *et al*., 2008; Satsuki, 1998).

Surface tension measurements for mixed anionic/cationic surfactants (C_mMES/C_nTAB) were also carried out in the same manner as single surfactants. Plots of surface tension against concentration for $C_{12}MES/C_nTAB$ systems are presented in Figure 3.12, $C_{14}MES/C_nTAB$ systems in Figure 3.13 and $C_{16}MES/C_nTAB$ in Figure 3.14.

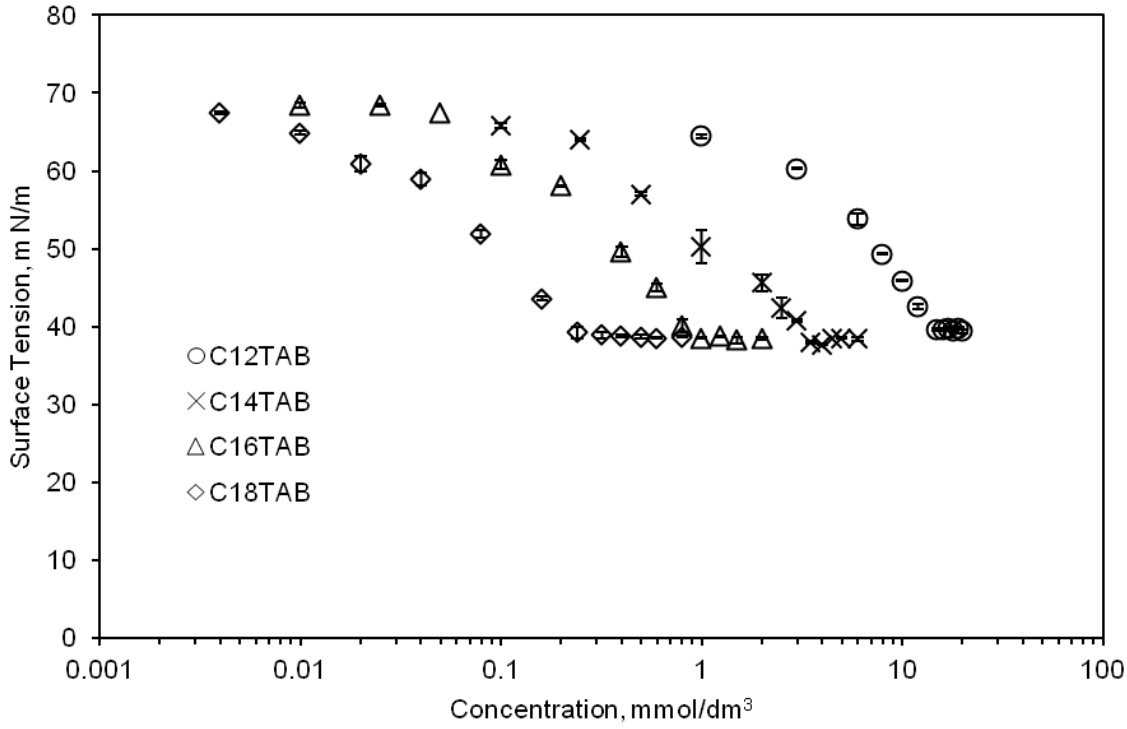


Figure 3.12 : Plots of surface tension against concentration for C_{12}TAB, C_{14}TAB and C_{16}TAB at 25˚C and C_{18}TAB at 35˚C.

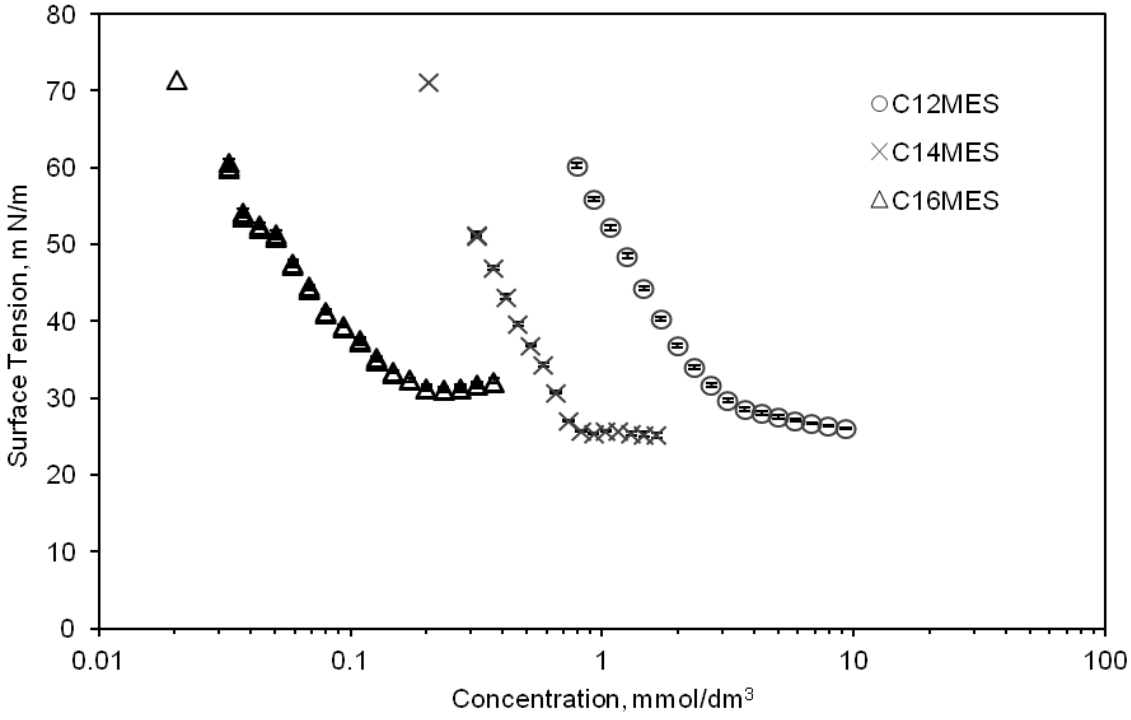


Figure 3.13 : Plots of surface tension against concentration for C_{12}MES and C_{14}MES at 25˚C and C_{16}MES at 35˚C.

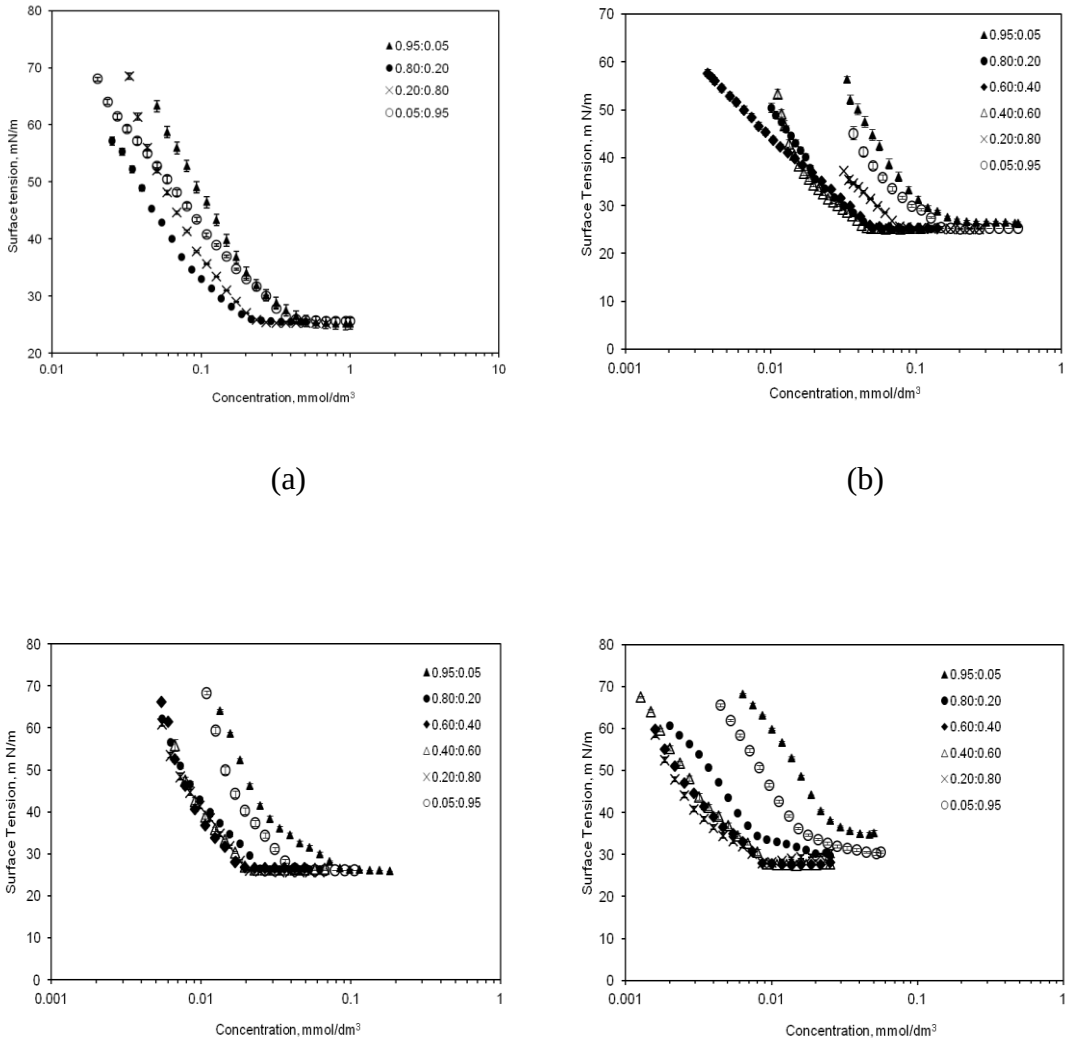


Figure 3.14 : Plots of surface tension against concentration for (a) C_{12}MES/C_{12}TAB, (b) C_{12}MES/C_{14}TAB and (c) C_{12}MES/C_{16}TAB at 25˚C and (d) C_{12}MES/C_{18}TAB at 35˚C.

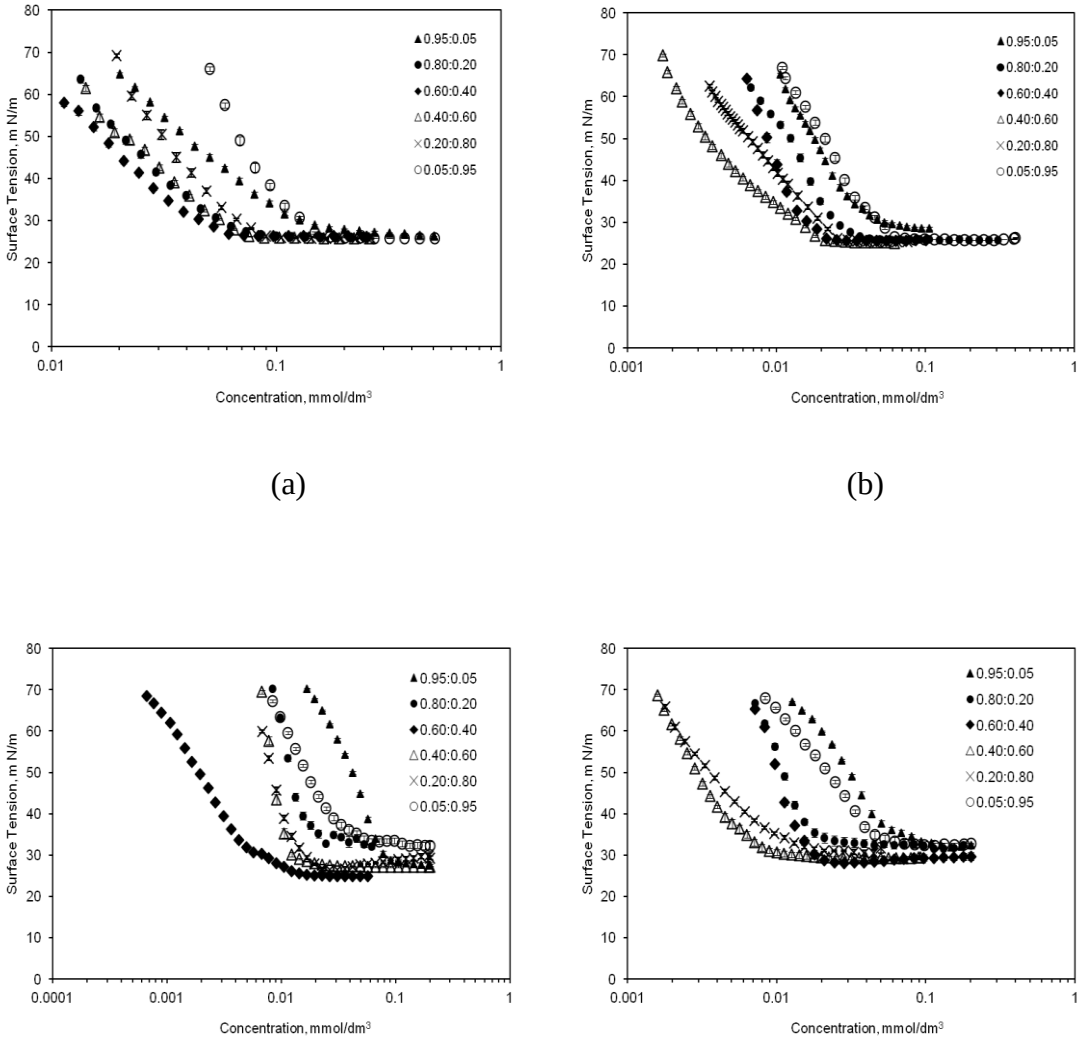
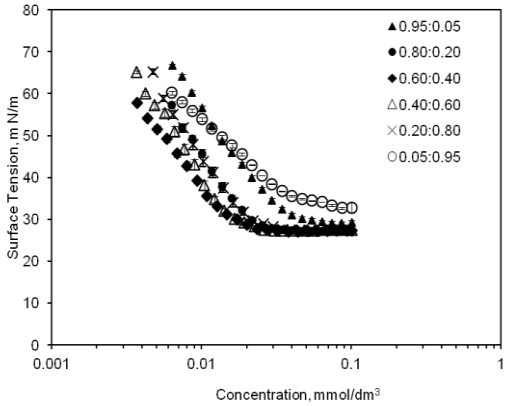
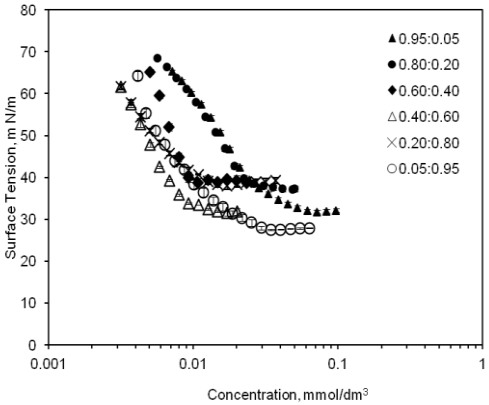


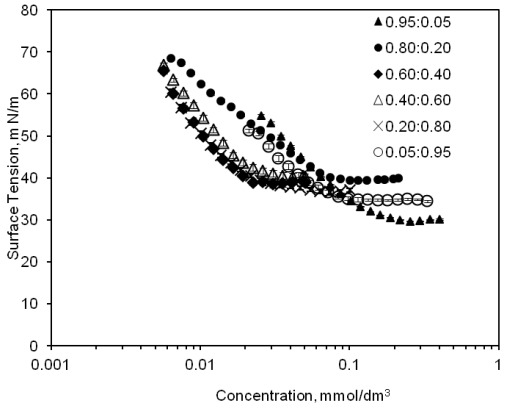
Figure 3.15 : Plots of surface tension against concentration for (a) C_{14}MES/C_{12}TAB, (b) C_{14}MES/C_{14}TAB and (c) C_{14}MES/C_{16}TAB at 25˚C and (d) C_{14}MES/C_{18}TAB at 35˚C.



(a)



(b)



(c)

Figure 3.16 : Plots of surface tension against concentration for (a) C_{16}MES/C_{12}TAB, (b) C_{16}MES/C_{16}TAB and (c) C_{16}MES/C_{18}TAB at 35˚C.

CMC values of different anionic/cationic mixtures (C_mMES/C_nTAB) are presented in Figure 3.15-3.17. Overall, the CMC values of most anionic/cationic (e.g. C_{12}MES/C_{12}TAB) mixtures were about 10-100 folds lower than the single surfactants, indicating a strong aggregating ability. As compared with other anionic/cationic mixtures such as alkyltrimethylammonium/cholate (Vinceković *et al*., 2006), tetradecyltrimethylammonium/dodecylbenzenesulfonate (Graciaa *et al*., 1989) and dodecyltrimethylammonium/dodecyl sulfate (Lucassen-Reynders, 1980), C_mMES/C_nTAB were found to exhibit intermediate performance in terms of CMC values as a result of interactions of the surfactant head groups (Rosen, 1989; Graciaa *et al*., 1989; Zhou and Rosen, 2003; Li and Zhao, 1992).

Theoretical ideal critical micelle concentration (CMC*) values for the anionic/cationic mixtures were also analyzed and compared with experimental CMC values. As depicted in Figure 3.15-3.17, the anionic/cationic mixtures signified non-ideality in mixed surfactant solutions as their CMC values are 10 to 100 fold lower than their CMC* values. These large negative deviations from CMC* indicated the presence of attractive forces and synergistic interactions between the oppositely charged surfactant molecules, which can be explained by the oppositely charged surfactant head groups acting as counterions to one another (Graciaa *et al*., 1989; Vora *et al*., 1999; Azum *et al*., 2008).

The effect of hydrocarbon chain length of both anionic (C_mMES) and cationic (C_nTAB) surfactants on the CMC values of the mixed systems was also been studied. It was observed that an increase in hydrocarbon chain length of C_nTAB led to a decreasing trend in CMC values of the mixed systems.

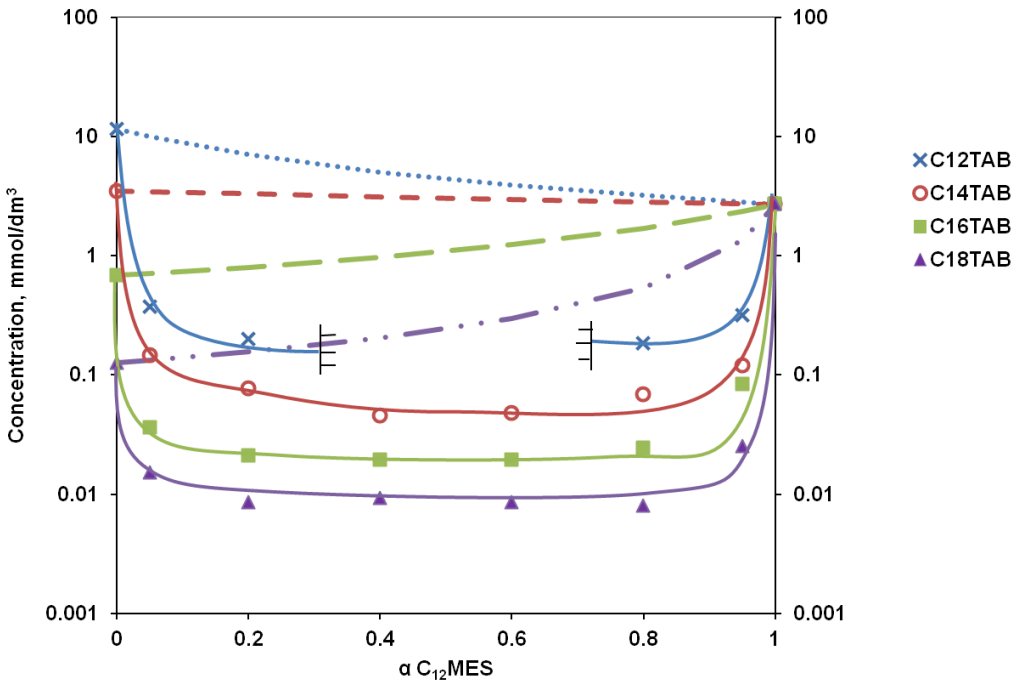


Figure 3.17 : Graph shows CMC of C_{12}MES, C_{12}TAB, C_{14}TAB, C_{16}TAB, C_{18}TAB and their mixtures. αC_{12}MES denotes mole fraction of C_{12}MES in the mixing ratio. Dotted lines represent ideal CMC for each system. All measurements are taken at 25℃ except for C_{18}TAB and its mixtures which are taken at 35℃.

At 0.2C_{12}MES/0.8C_nTAB mixing ratios CMC values of the mixtures dropped from 0.2mM to 0.08mM followed by 0.02mM and lastly 0.01mM for C_{12}TAB, C_{14}TAB, C_{16}TAB to C_{18}TAB, respectively. This was due to the increase in hydrophobicity that contributes to greater van der Waals forces between the hydrocarbon chains which conditions were favourable for the formation of micelle (Li and Zhao, 1992). Regardless of the mixing ratios of C_{12}MES in the C_{12}MES/C_nTAB mixed systems, CMC values of the mixtures were quite similar from 0.2C_{12}MES/0.8C_nTAB to 0.8C_{12}MES/0.2C_nTAB. However, CMC values for 0.4C_{12}MES/0.6C_{12}TAB and 0.6C_{12}MES/0.4 C_{12}TAB could not be determined due to precipitation problems even at very low concentration. The

high tendency to form precipitate at near equimolar mixing ratio was caused by the compatibility of the surfactant chain length.

CMC values of the C_{14}MES/C_nTAB mixtures (Figure 3.16) decrease as the hydrocarbon chain length of the cationic surfactants increases. However, the decrease in CMC values for C_{14}MES/C_nTAB mixtures at higher hydrocarbon chain length of cationic surfactant were relatively lesser due to thermodynamic reasons, as the entropy loss resulting from micellization of the surfactants becomes smaller (Jurašin *et al*., 2010). For C_{14}MES/C_{16}TAB and C_{14}MES/C_{18}TAB mixtures, their minimum CMC values were also found to be skewered to the left (cationic-rich region). As the chain length for both surfactants was different, the portion of the molecules above the height of adjacent molecules exhibited thermal motion. This thermal disturbance propagates along the chain length toward the head group of the molecule, causing an increase in the area/molecule (Patist *et al*., 1997). Therefore, an excess of cationic surfactants may help to stabilize this thermal motion in the mixed systems and may allow the mixed molecules to pack closer to each other.

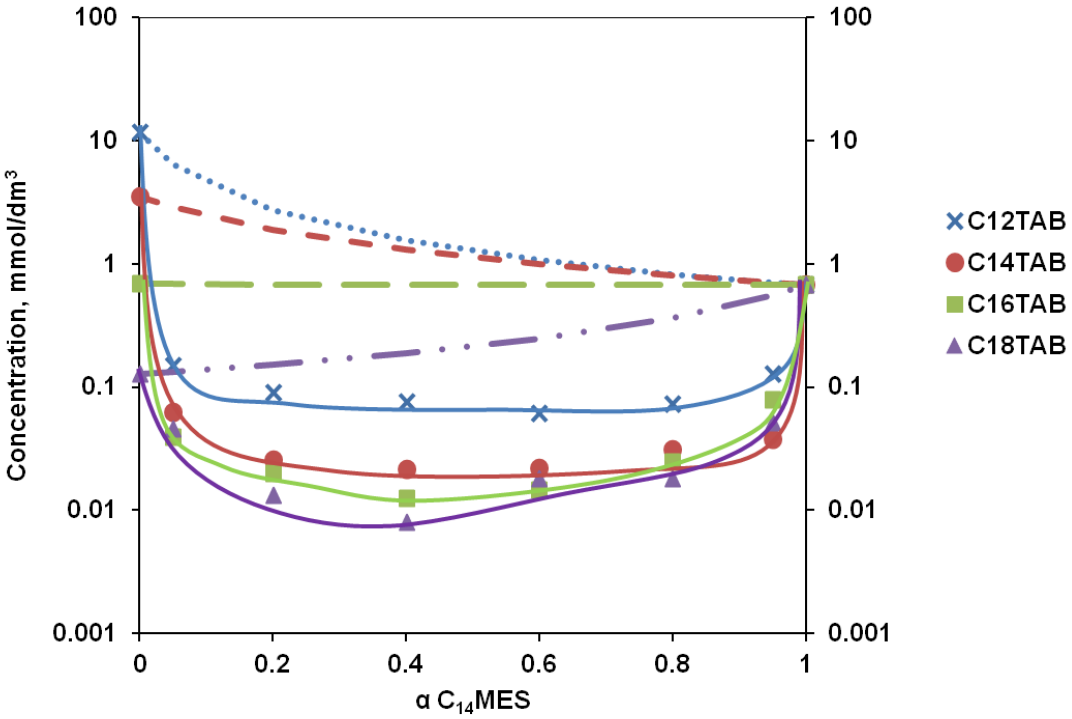


Figure 3.18 : Graph shows CMC of C_{14}MES, C_{12}TAB, C_{14}TAB, C_{16}TAB, C_{18}TAB and their mixtures. αC_{14}MES denotes mole fraction of C_{14}MES in the mixing ratio. Dotted lines represent ideal CMC for each system. All measurements are taken at 25℃ except for C_{18}TAB and its mixtures which are taken at 35℃.

As for C_{16}MES/C_nTAB series, the surface tension measurements were carried out at 35˚C due to the high Krafft point temperature of C_{16}MES. Surface properties of C_{16}MES/C_{14}TAB mixtures could not be carried due to precipitation problems that persist over a wide range of mixing ratios (0.2 C_{16}MES/0.8C_{14}TAB to 0.8C_{16}MES/0.2C_{14}TAB) at very low concentration. From Figure 3.17, the decrement in CMC values of the C_{16}MES/C_nTAB mixtures as the hydrocarbon chain length increases was very low. Besides that, the CMC values of C_{16}MES/C_{18}TAB mixtures were found to be unexpectedly higher than the C_{16}MES/C_{12}TAB and C_{16}MES/C_{16}TAB series. Such a phenomenon could be due to the high hydrophobicity of the hydrocarbon chain length

in the mixed systems which decreased its solubility and packing of the molecules in the micelles becomes difficult (Zhou and Rosen, 2003).

The CMC values for both C_{16}MES/C_{16}TAB and C_{16}MES/C_{18}TAB series were also skewed to the left (cationic-rich region), which were similar to both C_{14}MES/C_{16}TAB and C_{14}MES/C_{18}TAB mixtures as described earlier.

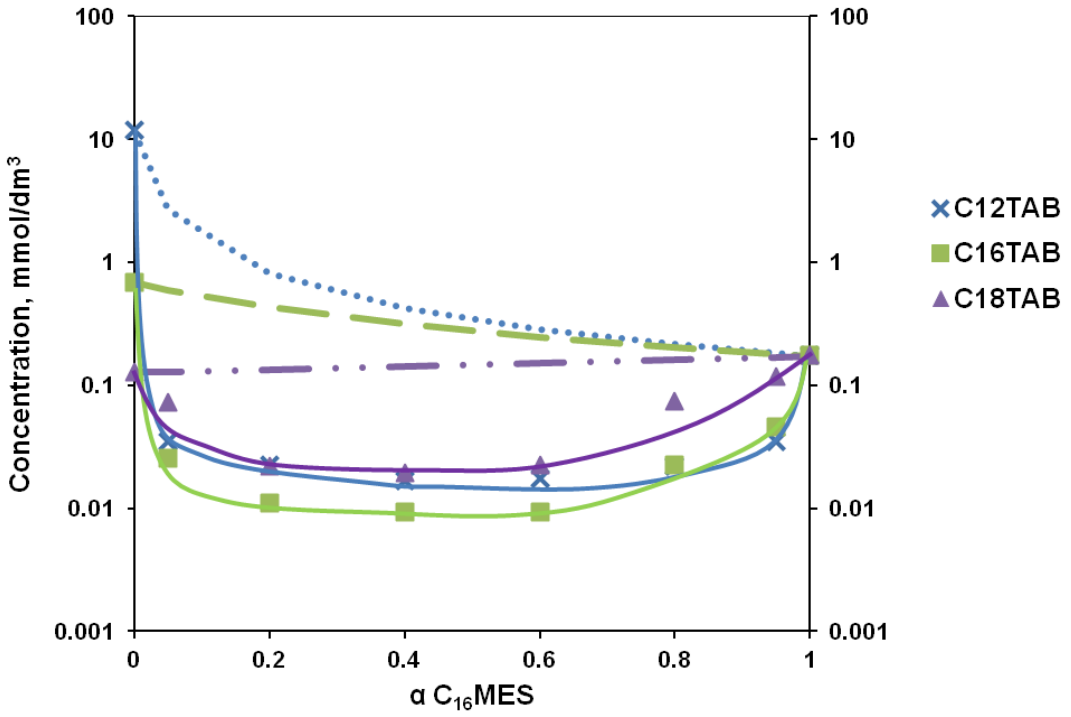
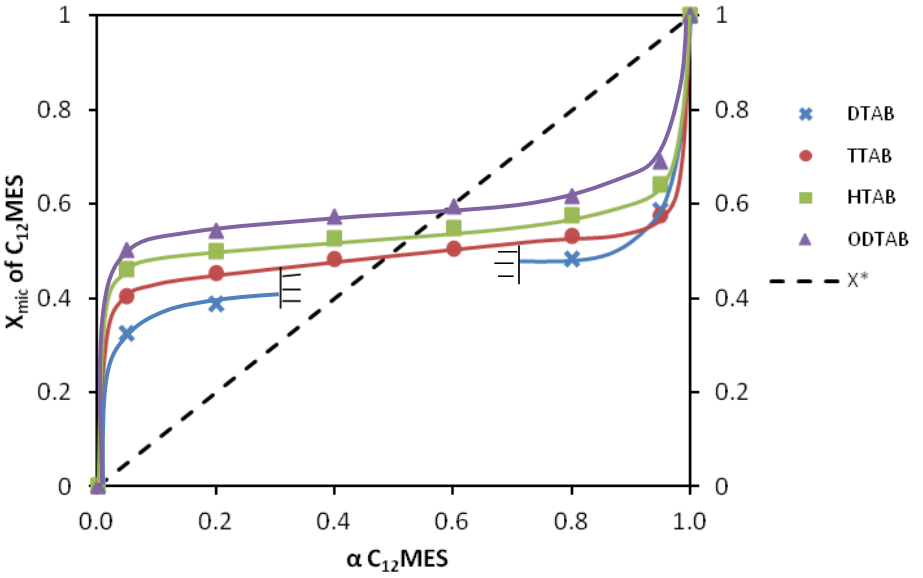


Figure 3.19 : Graph shows CMC of C_{16}MES, C_{12}TAB, C_{14}TAB, C_{16}TAB, C_{18}TAB and their mixtures. αC_{16}MES denotes mole fraction of C_{16}MES in the mixing ratio. Dotted lines represent ideal CMC for each system. All measurements are taken at 35℃.

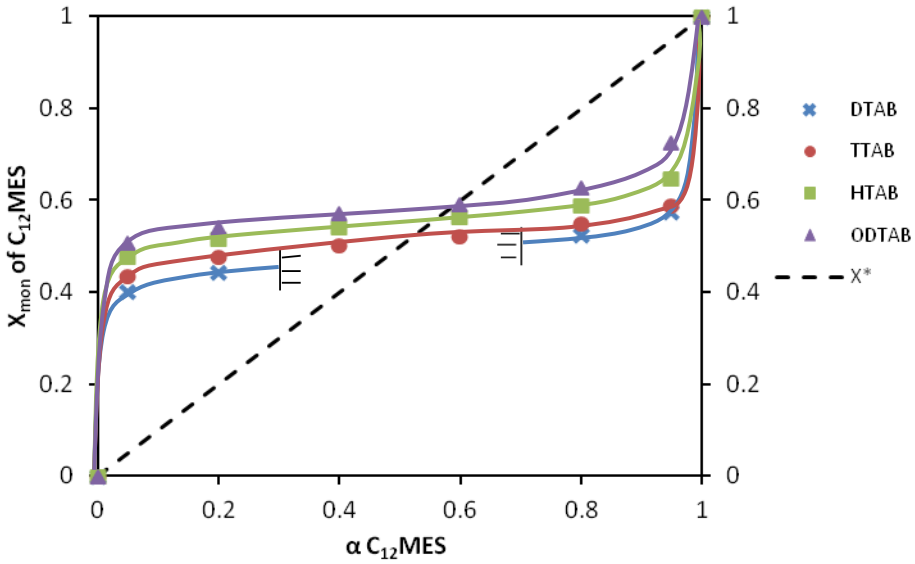
In addition, the effect of hydrocarbon chain length of C_nTAB on CMC values was found to be more significant than the C_mMES surfactants. This was due to the large head group size of C_mMES head group compared to those of C_nTAB. Therefore, the additional CH_2 to the hydrocarbon chain length was more significant in C_nTAB surfactants.

3.2.1.2 Interactions of the Anionic/Cationic Surfactant Molecules

In the present study, interactions between the oppositely charged surfactant molecules were analyzed according to Rubingh's regular solution theory (Rosen, 1989). The actual composition and interaction parameters for the mixed systems at air/water interface monolayer and in the mixed micelle were evaluated using eq. 2-5 and presented in Figure 3.18-3.20. In the mixed ratios range between $0.2C_mMES/0.8C_nTAB$ to $0.8C_mMES/0.2C_nTAB$, the micelle compositions of the mixed surfactants were very close to equimolarity. A similar trend was also observed in the composition of mixed monolayers. Such rare behavior could be explained by the strong attractive forces between the oppositely charged surfactant molecules in which the molecules tend to attract to different charged surfactant molecules instead of similar charged surfactant molecules (Graciaa *et al*., 1989). This behavior is normal for anionic/cationic mixtures as studies from other researchers also showed similar trends (Graciaa *et al*., 1989; Zhou and Rosen, 2003; Rosen and Zhou, 2001). However, for the $C_{16}MES/C_{18}TAB$ system, the composition of $C_{16}MES$ in the mixture seemed to deviate further from equimolarity. This could be due to the long hydrocarbon chain length of the surfactants was inhibiting to some degree its self-assembly (Zhou and Rosen, 2003)

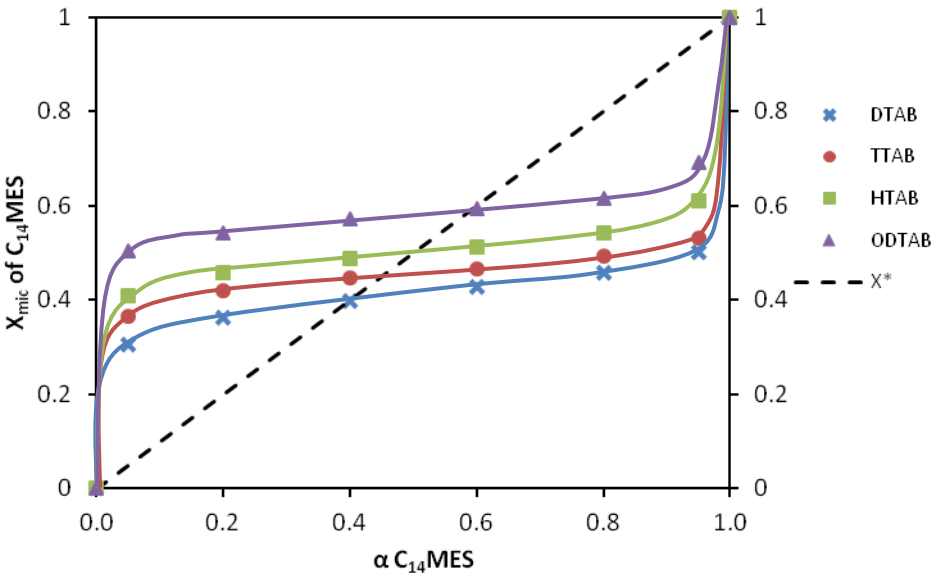


(a)

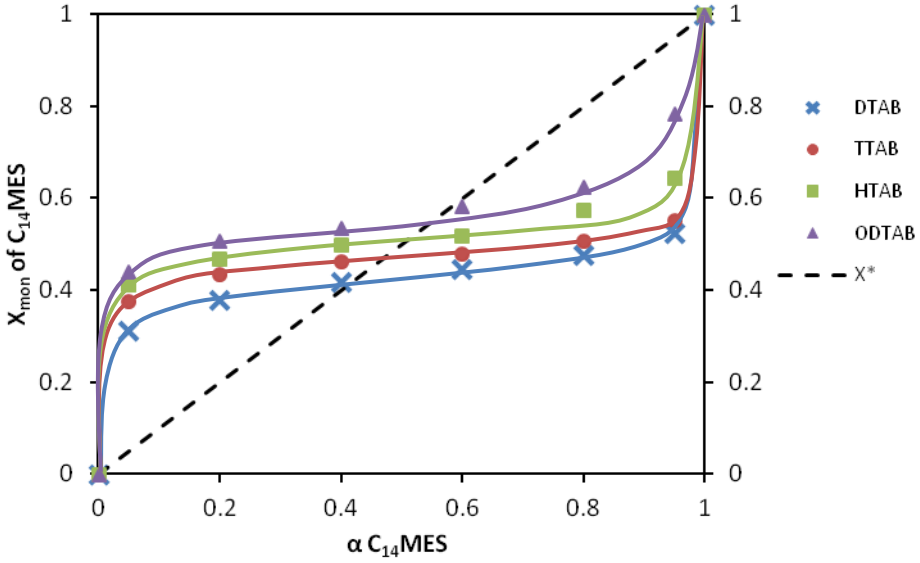


(b)

Figure 3.20 : Mole fraction of C_{12}MES in (a) micelle and (b) monolayer. Dotted line represent ideal mole fraction of C_{12}MES.

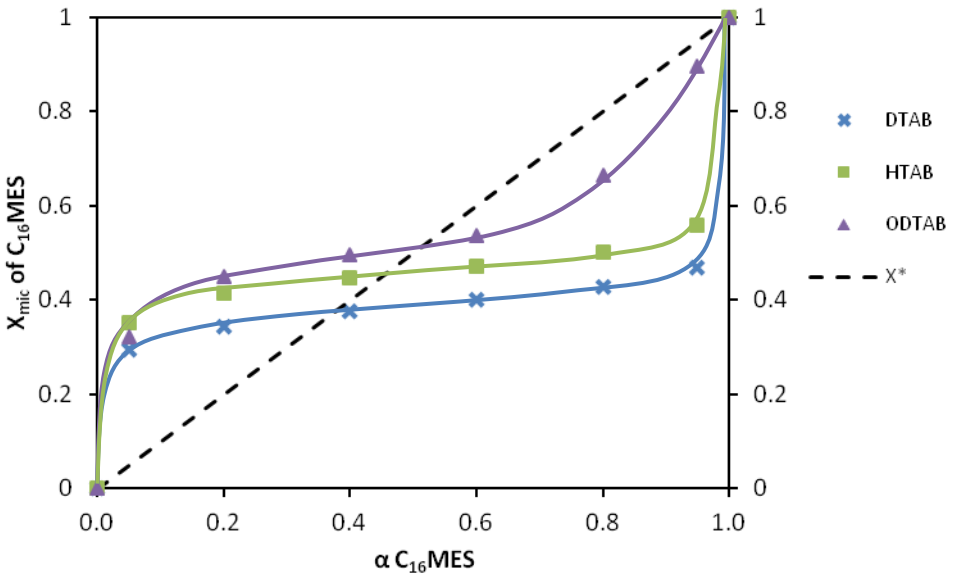


(a)

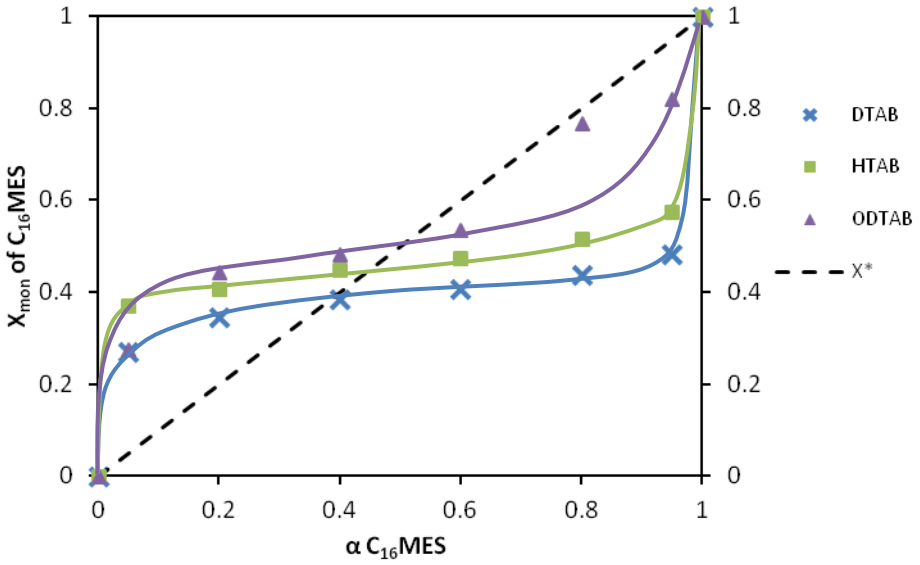


(b)

Figure 3.21 : Mole fraction of C_{14}MES in (a) micelle and (b) monolayer. Dotted line represent ideal mole fraction of C_{14}MES.



(a)



(b)

Figure 3.22 : Mole fraction of C_{16}MES in (a) micelle and (b) monolayer. Dotted line represent ideal mole fraction of C_{16}MES.

The interaction parameter in the mixed micelle (β_{mic}) and interaction parameter at the liquid/air interface (β_{mon}) values of the mixed C_mMES/C_nTAB systems calculated based on Rubingh's regular solution theory are listed and compared in Table 3.2-3.4. In general, all the β values for mixed surfactants were highly negative ranging from -10 to -20. This indicated strong attractive interactions among the anionic and cationic surfactant molecules in mixed micelles and mixed monolayer (Sohrabi *et al*., 2008; Li and Liu, 1994; Dragčević *et al*., 1995; Zhou and Rosen, 2003; Azum *et al*., 2008; Rosen and Zhou, 2001; Tomašić *et al*., 1999). Other anionic/cationic systems also showed high negative values of both β_{mic} and β_{mon}. For instance, β values of tetradecyltrimethylammonium-dodecylbenzenesulfonate system obtained from Graciaa and co-workers (1989) was approximately -24.1, where as Sohrabi and co-workers (2008) had β_{mon} values for their anionic/cationic systems in the range of -9 to -22 and β_{mic} values between -5 and -10.

Among the C_mMES/C_nTAB mixtures, C_{16}MES/C_{18}TAB systems exhibited exceptionally low β_{mic} (-3 to -8.5) and β_{mon} (-4 to -9) values, indicating weaker synergistic interactions between the oppositely charged surfactant molecules. This suggested that the hydrophobic tails of the surfactants were easily accommodated at the liquid/air interface than in the interior of a space-deprived micelle. A similar behaviour was also reported in the works of Sohrabi *et al*. (2008) and Zhou and Rosen (2003).

For the C_{12}MES/C_nTAB series, a slight increase in the β_{mic} values was observed as the chain length of the C_nTAB increases. As the hydrocarbon chain length increases, van der Waals forces between the hydrocarbon chains also increase. Thus, the stronger synergistic interactions between the C_{12}MES and C_nTAB surfactant molecules lead to a high β_{mic} value. Meanwhile, for the C_{14}MES/C_nTAB and C_{16}MES/C_nTAB series, a maximum β_{mic} value was observed at C_{14}MES/ C_{14}TAB and C_{16}MES/C_{12}TAB, respectively. This may be due to the packing of the surfactant molecules in the mixed micelle is the most optimum at that combination.

The values of β_{mic} were generally not the same as the value of β_{mon} for two similar surfactants under the same condition. In most cases, β_{mon} values were more negative than β_{mic} values which mean that the repulsive electrostatic forces between surfactant molecules at the mixed monolayer were more effectively reduced than in the mixed micelle. But there were also some extraordinary situations in which interactions of surfactant molecules were stronger in the mixed micelle than at the liquid/air interface. In the case of C_{12}MES/C_{12}TAB and C_{16}MES/ C_{16}TAB mixtures, it could be simply due to the large composition of one surfactant over another surfactant which greatly affects the molecular homogeneity and the constancy of the ionic strength of the solution (Zhou and Rosen, 2003). As for the case of C_{12}MES/C_{18}TAB and C_{14}MES/C_{18}TAB mixtures, positive values of (β_{mon} - β_{mic}) could be due to the incompatible hydrophobic chain lengths of anionic and cationic surfactants. The surfactants were able to pack closer in the curved geometry in a micelle rather than in a planar geometry of the liquid/air interface monolayer (Sohrabi *et al*., 2008)

The values of the activity coefficients, g_1 and g_2 which are calculated using eq. 6 and 7 are also listed in Table 3.2-3.4. Both the g_1 and g_2 values obtained were very small and their summation was less than unity. This further confirmed that the C_mMES/C_nTAB mixed systems were non-ideal (Azum *et al*., 2008; Tomašić *et al*., 1999). The negative values of ΔG°_{ex} obtained, again indicated that the mixed micelles formed were more stable than the micelles of individual surfactants (Azum *et al*., 2008). Similar to the β_{mic} trend, the most negative values of ΔG°_{ex} were found to be for C_{12}MES/C_{18}TAB, C_{14}MES/C_{14}TAB and C_{16}MES/C_{12}TAB in their respective C_mMES series.

Table 3.2 : Interaction parameter in micelle, β_{mic} , interaction parameter in monolayer, β_{mon}, activity coefficient of $C_{12}MES$, g_1, activity coefficient of C_nTAB, g_2 and excess Gibbs free energy of mixing, ΔG°_{ex} for $C_{12}MES/C_nTAB$ and their mixtures at different mixing ratios of $C_{12}MES$, α_1.

α_1	β_{mic}	β_{mon}	$\beta_{mon} - \beta_{mic}$	g_1	g_2	ΔG°_{ex} kJ/mol	ΔG^{M}_{E} kJ/mol	ΔG^{σ}_{E} kJ/mol
$C_{12}MES$ - $C_{12}TAB$								
0.00	-	-	-	-	-	-	-	-
0.05	-12.89	-15.33	-2.44	0.0049	0.2951	-7.29	-7.29	-9.12
0.20	-13.71	-14.61	-0.90	0.0089	0.1486	-8.21	-8.21	-8.93
0.40	-	-	-	-	-	-	-	-
0.60	-	-	-	-	-	-	-	-
0.80	-14.54	-15.73	-1.19	0.0263	0.0407	-9.00	-9.00	-9.72
0.95	-14.67	-14.45	0.22	0.0442	0.0020	-9.02	-9.02	-8.76
1.00	-	-	-	-	-	-	-	-
Average	**-13.95**	**-15.03**	**-1.08**			**-8.38**	**-8.38**	**-9.13**
$C_{12}MES$ - $C_{14}TAB$								
0.00	-	-	-	-	-	-	-	-
0.05	-14.86	-16.71	-1.84	0.0055	0.1292	-8.87	-8.87	-10.17
0.20	-15.51	-17.68	-2.17	0.0100	0.0617	-9.52	-9.52	-10.93
0.40	-16.93	-17.88	-0.96	0.0143	0.0374	-10.47	-10.47	-11.08
0.60	-16.74	-18.97	-2.23	0.0220	0.0282	-10.37	-10.37	-11.73
0.80	-16.05	-17.77	-1.72	0.0349	0.0189	-9.90	-9.90	-10.91
0.95	-15.90	-17.32	-1.42	0.0814	0.0114	-9.63	-9.63	-10.38
1.00	-	-	-	-	-	-	-	-
Average	**-16.00**	**-17.72**	**-1.72**			**-9.79**	**-9.79**	**-10.87**
$C_{12}MES$ - $C_{16}TAB$								
0.00	-	-	-	-	-	-	-	-
0.05	-17.69	-17.15	0.54	0.0070	0.0409	-10.89	-10.89	-10.60
0.20	-17.54	-17.53	0.01	0.0127	0.0187	-10.86	-10.86	-10.85
0.40	-16.99	-16.91	0.08	0.0223	0.0137	-10.50	-10.50	-10.40
0.60	-16.88	-16.74	0.14	0.0320	0.0096	-10.36	-10.36	-10.20
0.80	-16.52	-16.94	-0.42	0.0511	0.0064	-10.00	-10.00	-10.16
0.95	-13.23	-14.85	-1.62	0.1849	0.0065	-7.54	-7.54	-8.41
1.00	-	-	-	-	-	-	-	-
Average	**-16.47**	**-16.69**	**-0.21**			**-10.02**	**-10.02**	**-10.10**
$C_{12}MES$ - $C_{18}TAB$								
0.00	-	-	-	-	-	-	-	-
0.05	-17.93	-17.00	0.93	0.0091	0.0158	-11.48	-11.48	-10.88
0.20	-17.64	-19.78	-2.14	0.0190	0.0082	-11.21	-11.21	-12.57
0.40	-16.29	-17.70	-1.42	0.0391	0.0073	-10.21	-10.21	-11.11
0.60	-16.32	-17.85	-1.53	0.0521	0.0046	-10.07	-10.07	-11.05
0.80	-17.05	-16.00	1.05	0.0619	0.0022	-10.32	-10.32	-9.58
0.95	-13.61	-11.60	2.01	0.2094	0.0021	-7.44	-7.44	-5.93
1.00	-	-	-	-	-	-	-	-
Average	**-16.47**	**-16.65**	**-0.18**			**-10.12**	**-10.12**	**-10.19**

Table 3.3 : Interaction parameter in micelle, β_{mic} , interaction parameter in monolayer, β_{mon}, activity coefficient of C_{14}MES, g_1, activity coefficient of C_nTAB, g_2 and excess Gibbs free energy of mixing, ΔG°_{ex} for C_{14}MES/C_nTAB and their mixtures at different mixing ratios of C_{14}MES, α_1.

α_1	β_{mic}	β_{mon}	$\beta_{mon} - \beta_{mic}$	g_1	g_2	ΔG°_{ex} kJ/mol	ΔG_E^M kJ/mol	ΔG_E^{σ} kJ/mol
C_{14}MES - C_{12}TAB								
0.00	-	-	-	-	-	-	-	-
0.05	-12.83	-11.43	1.39	0.0021	0.3014	-6.75	-6.75	-6.07
0.20	-13.48	-12.69	0.80	0.0042	0.1685	-7.73	-7.73	-7.40
0.40	-13.86	-13.62	0.24	0.0065	0.1121	-8.23	-8.23	-8.21
0.60	-15.02	-14.71	0.31	0.0073	0.0638	-9.11	-9.11	-9.01
0.80	-15.36	-14.98	0.38	0.0093	0.0345	-9.44	-9.44	-9.26
0.95	-15.71	-14.59	1.12	0.0208	0.0190	-9.73	-9.73	-9.02
1.00	-	-	-	-	-	-	-	-
Average	**-14.38**	**-13.67**	**0.71**			**-8.50**	**-8.50**	**-8.16**
C_{14}MES - C_{14}TAB								
0.00	-	-	-	-	-	-	-	-
0.05	-14.90	-14.37	0.53	0.0024	0.1383	-8.55	-8.55	-8.36
0.20	-16.76	-17.05	-0.28	0.0035	0.0527	-10.11	-10.11	-10.38
0.40	-16.98	-18.79	-1.81	0.0054	0.0342	-10.40	-10.40	-11.58
0.60	-17.03	-16.30	0.73	0.0079	0.0243	-10.50	-10.50	-10.08
0.80	-16.48	-15.08	1.40	0.0144	0.0183	-10.20	-10.20	-9.34
0.95	-18.08	-15.97	2.11	0.0191	0.0060	-11.15	-11.15	-9.79
1.00	-	-	-	-	-	-	-	-
Average	**-16.71**	**-16.26**	**0.45**			**-10.15**	**-10.15**	**-9.92**
C_{14}MES - C_{16}TAB								
0.00	-	-	-	-	-	-	-	-
0.05	-14.19	-12.57	1.62	0.0082	0.1084	-8.50	-8.50	-7.54
0.20	-14.94	-13.91	1.03	0.0146	0.0501	-9.19	-9.19	-8.58
0.40	-16.08	-13.78	2.30	0.0149	0.0216	-9.95	-9.95	-8.53
0.60	-15.46	-18.68	-3.21	0.0248	0.0176	-9.57	-9.57	-11.55
0.80	-14.02	-9.69	4.33	0.0458	0.0138	-8.62	-8.62	-5.87
0.95	-11.32	-9.30	2.02	0.1779	0.0149	-6.68	-6.68	-5.27
1.00	-	-	-	-	-	-	-	-
Average	**-14.34**	**-12.99**	**1.35**			**-8.75**	**-8.75**	**-7.89**
C_{14}MES - C_{18}TAB								
0.00	-	-	-	-	-	-	-	-
0.05	-10.62	-9.19	1.43	0.0399	0.1171	-6.73	-6.73	-5.80
0.20	-13.29	-13.74	-0.45	0.0407	0.0319	-8.50	-8.50	-8.79
0.40	-14.39	-14.53	-0.14	0.0466	0.0154	-9.16	-9.16	-9.25
0.60	-10.90	-10.12	0.78	0.1464	0.0255	-6.80	-6.80	-6.31
0.80	-11.35	-9.91	1.43	0.1844	0.0139	-6.89	-6.89	-5.95
0.95	-8.28	-5.71	2.57	0.5251	0.0135	-4.26	-4.26	-2.48
1.00	-	-	-	-	-	-	-	-
Average	**-11.47**	**-10.53**	**0.94**			**-7.06**	**-7.06**	**-6.43**

Table 3.4 : Interaction parameter in micelle, β_{mic} , interaction parameter in monolayer, β_{mon}, activity coefficient of $C_{16}MES$, g_1, activity coefficient of C_nTAB, g_2 and excess Gibbs free energy of mixing, ΔG°_{ex} for $C_{16}MES/C_nTAB$ and their mixtures at different mixing ratios of $C_{16}MES$, α_1.

α_1	β_{mic}	β_{mon}	$\beta_{mon} - \beta_{mic}$	g_1	g_2	ΔG°_{ex} kJ/mol	ΔG_E^M kJ/mol	ΔG_E^{σ} kJ/mol
$C_{16}MES$ - $C_{12}TAB$								
0.00	-	-	-	-	-	-	-	-
0.05	-15.24	-12.12	3.12	0.0005	0.3082	-8.10	-8.10	-6.13
0.20	-15.78	-14.16	1.61	0.0011	0.1799	-9.11	-9.11	-8.21
0.40	-16.75	-15.10	1.65	0.0015	0.1068	-10.07	-10.07	-9.14
0.60	-16.96	-15.29	1.67	0.0002	0.0095	-10.42	-10.42	-9.46
0.80	-17.20	-15.50	-1.70	0.0035	0.0507	-10.77	-10.77	-9.76
0.95	-18.12	-15.73	2.39	0.0060	0.0217	-11.56	-11.56	-10.05
1.00	-	-	-	-	-	-	-	-
Average	**-16.68**	**-14.65**	**2.03**			**-10.00**	**-10.00**	**-8.79**
$C_{16}MES$ – $C_{14}TAB$*								
$C_{16}MES$ - $C_{16}TAB$								
0.00	-	-	-	-	-	-	-	-
0.05	-12.46	-13.30	-0.84	0.0054	0.2473	-7.27	-7.27	-7.93
0.20	-14.25	-11.18	3.06	0.0078	0.0992	-8.86	-8.86	-6.91
0.40	-14.32	-12.52	1.80	0.0124	0.0674	-9.06	-9.06	-7.93
0.60	-14.47	-10.80	3.66	0.0175	0.0470	-9.23	-9.23	-6.90
0.80	-11.79	-8.71	3.08	0.0533	0.0602	-7.55	-7.55	-5.57
0.95	-11.25	-10.55	0.70	0.1131	0.0342	-7.10	-7.10	-6.60
1.00	-	-	-	-	-	-	-	-
Average	**-13.09**	**-11.18**	**1.91**			**-8.18**	**-8.18**	**-6.98**
$C_{16}MES$ - $C_{18}TAB$								
0.00	-	-	-	-	-	-	-	-
0.05	-5.27	-4.03	1.25	0.0948	0.6555	-2.94	-2.94	-2.05
0.20	-8.46	-9.06	-0.60	0.0779	0.2095	-5.36	-5.36	-5.73
0.40	-8.23	-4.85	3.38	0.1248	0.1526	-5.27	-5.27	-3.10
0.60	-7.56	-6.05	1.52	0.1485	0.0946	-4.81	-4.81	-3.85
0.80	-3.10	-0.66	2.45	0.7038	0.2553	-1.77	-1.77	-0.30
0.95	-1.38	-2.47	-1.09	0.9853	0.3304	-0.33	-0.33	-0.93
1.00	-	-	-	-	-	-	-	-
Average	**-5.67**	**-4.52**	**1.15**			**-3.41**	**-3.41**	**-2.66**

* Surface measurements for $C_{16}MES$/ $C_{14}TAB$ mixed systems were unable to be conducted due to precipitations problems even at low concentrations. Therefore, calculations were omitted for this system.

3.2.1.3 Properties at the Air/Water Interface

Surfactants in solution will orientate themselves spontaneously at the air-water interface and disturbs the orientation of water molecules at the surface. This will then lead to a decrease in surface tension. The increase in adsorption effectiveness for the mixed surfactants was due to the reduction in the electrostatic repulsions between the headgroups. Thus, the surfactant molecules can orientate themselves closer to each other, allowing more molecules to adsorb at the interface. Actual amounts of surfactant molecules adsorbed on the air/water interface at various concentrations can be calculated with the Gibbs adsorption equation for aqueous surfactant mixtures (Murphy and Taggart, 2002). The calculated surface excess concentration, Γ_{max} and minimum area per molecule, A_{min} are listed in Table 3.5-3.7 for C_{12}MES/C_nTAB, C_{14}MES/C_nTAB and C_{16}MES/C_nTAB mixtures, respectively. All mixed systems have a higher Γ_{max} than the single surfactants. This showed that the effectiveness of the surfactant mixtures to adsorb on the liquid/air interface was much higher than the single surfactants.

The calculated values of A_{min} were also in agreement with Γ_{max} in which the A_{min} values for mixed surfactants were lower as compared to the single surfactants. This showed that the mixed surfactant molecules were more densely packed at the liquid/air interface due to ion-pairing. This characteristic is yet another piece of evidence of the presence of synergistic interactions between the oppositely charged surfactant molecules (Li and Liu, 1994; Dragčević *et al*., 1995; Harkot and Janczuk, 2008; Azum *et al*., 2008; Tomašić *et al*., 1999). However, it is difficult to compare Γ_{max} and A_{min} values at different mixing ratios of the same mixed systems because the differences in Γ_{max} were small and insignificant.

Table 3.5 : Surface excess concentration, Γ_{max} and minimum area per molecule, A_{min} for $C_{12}MES/C_nTAB$ and their mixtures at different mixing ratios of $C_{12}MES$, α_1.

α_1	Γ_{max}, x 10^{-6} mol/m^2	A_{min}, nm
$C_{12}MES$ - $C_{12}TAB$		
0.00	1.45	1.14
0.05	2.42	0.69
0.20	3.28	0.51
0.40	-	-
0.60	-	-
0.80	2.72	0.61
0.95	2.42	0.69
1.00	1.98	0.84
$C_{12}MES$ - $C_{14}TAB$		
0.00	1.32	1.26
0.05	2.34	0.71
0.20	2.24	0.74
0.40	2.13	0.78
0.60	2.13	0.78
0.80	2.46	0.68
0.95	2.16	0.77
1.00	1.98	0.84
$C_{12}MES$ - $C_{16}TAB$		
0.00	1.16	1.43
0.05	3.74	0.44
0.20	3.93	0.42
0.40	3.71	0.45
0.60	3.61	0.46
0.80	3.39	0.49
0.95	2.05	0.81
1.00	1.98	0.84
$C_{12}MES$ - $C_{18}TAB$		
0.00	0.98	1.70
0.05	4.15	0.40
0.20	2.05	0.81
0.40	2.46	0.68
0.60	3.03	0.55
0.80	3.68	0.45
0.95	3.87	0.43
1.00	1.98	0.84

Table 3.6 : Surface excess concentration, Γ_{max} and minimum area per molecule, A_{min} for $C_{14}MES/C_nTAB$ and their mixtures at different mixing ratios of $C_{14}MES$, α_1.

α_1	Γ_{max}, x 10^{-6} mol/m^2	A_{min}, nm
$C_{14}MES$ - $C_{12}TAB$		
0.00	1.45	1.14
0.05	4.91	0.34
0.20	3.63	0.46
0.40	3.40	0.49
0.60	3.05	0.55
0.80	3.48	0.48
0.95	3.22	0.52
1.00	2.39	0.84
$C_{14}MES$ - $C_{14}TAB$		
0.00	1.32	1.26
0.05	3.01	0.55
0.20	3.18	0.52
0.40	2.08	0.80
0.60	3.05	0.54
0.80	4.87	0.34
0.95	4.44	0.37
1.00	2.39	0.84
$C_{14}MES$ - $C_{16}TAB$		
0.00	1.16	1.43
0.05	2.36	0.70
0.20	2.20	0.76
0.40	6.58	0.25
0.60	1.13	1.47
0.80	3.28	0.51
0.95	5.48	0.30
1.00	2.39	0.84
$C_{14}MES$ - $C_{18}TAB$		
0.00	0.98	1.70
0.05	3.53	0.47
0.20	1.81	0.92
0.40	2.54	0.65
0.60	5.86	0.28
0.80	6.17	0.27
0.95	4.29	0.39
1.00	2.39	0.84

Table 3.7 : Surface excess concentration, Γ_{max} and minimum area per molecule, A_{min} for $C_{16}MES/C_nTAB$ and their mixtures at different mixing ratios of $C_{16}MES$, α_1.

α_1	Γ_{max}, x 10^{-6} mol/m^2	A_{min}, nm
$C_{16}MES$ - $C_{12}TAB$		
0.00	1.45	1.14
0.05	2.39	0.70
0.20	3.53	0.47
0.40	4.03	0.41
0.60	3.20	0.52
0.80	3.78	0.44
0.95	3.16	0.53
1.00	1.35	1.23
$C_{16}MES$ – $C_{14}TAB$*		
$C_{16}MES$ - $C_{16}TAB$		
0.00	1.16	1.43
0.05	1.69	0.98
0.20	3.14	0.53
0.40	3.28	0.51
0.60	7.22	0.23
0.80	4.04	0.41
0.95	2.09	0.79
1.00	1.35	1.23
$C_{16}MES$ - $C_{18}TAB$		
0.00	1.01	1.64
0.05	2.19	0.76
0.20	2.33	0.71
0.40	3.25	0.51
0.60	3.13	0.53
0.80	1.92	0.87
0.95	2.55	0.65
1.00	1.35	1.23

* Surface measurements for $C_{16}MES$/ $C_{14}TAB$ mixed systems were unable to be conducted due to precipitations problems even at low concentrations. Therefore, calculations were omitted for this system.

3.2.2 Catanionic Surfactants

A large number of studies have been done on the physical properties of anionic/cationic mixed surfactants (also known as catanionic mixtures). It has been proven time and again that this oppositely charged mixed surfactant systems exhibit superior surface activities as compared to single surfactants and other types of binary mixtures (anionic/anionic, cationic/cationic, nonionic/anionic and etc.). However, there are few studies reported on the surface tension measurement of a true catanionic surfactant. This is due to the fact that catanionic surfactants have a low solubility in water, which makes surface tension measurement difficult to be carried out. Nevertheless, certain combinations of anionic and cationic surfactants are more readily soluble in water than others. For instance, Chen and co-workers (2004) reported that C_nSO_3–C_nN mixtures were unexpectedly much more soluble in water than C_nSO_4–C_nN mixtures. Although the chemical difference between alkyl sulfate and alkyl sulfonate is just one oxygen atom, their catanionic surfactant mixtures are quite different in terms of solution properties, phase behavior and aggregate properties, which can be attributed to their intermolecular interactions. In addition to that, surface properties of cetyltrimethylammonium octylsulfonate (TaSO) were also successfully measured by Fernandes and co-workers (2010). Another reason that contributes to the high solubility of TaSO is the highly mismatched hydrocarbon chain length.

In our studies, most of the C_mMES.C_nTAB catanionic surfactants produced were highly soluble in water at 30±0.1˚C. Therefore, their behaviour at the air/water interface was studied using surface tensiometer. The difference in hydrocarbon chain length of C_mMES.C_nTAB catanionic surfactants was observed and compared.

3.2.2.1 Critical Micelles Concentration (CMC)

Surface tension measurements of selected single and catanionic surfactants system in diluted solution are shown in Figure 3.21 – 3.23. CMC values that were obtained from the point where the two lines intersect in the surface tension, γ against concentration, M plot are listed in Table 3.8. All C_{12}MES.C_nTAB and C_{14}MES.C_nTAB catanionic surfactants exhibit a CMC. However for C_{16}MES.C_nTAB series, only C_{16}MES.C_{12}TAB system showed a CMC. This was due to the low solubility contributed by its long hydrocarbon chain length. CMC values of the catanionic surfactants were very low. Such phenomena were also observed in other studies and were related to the high synergistic interactions between the oppositely charged surfactant molecules (Graciaa *et al.*, 1989; Vora *et al*., 1999; Azum *et al*., 2008)

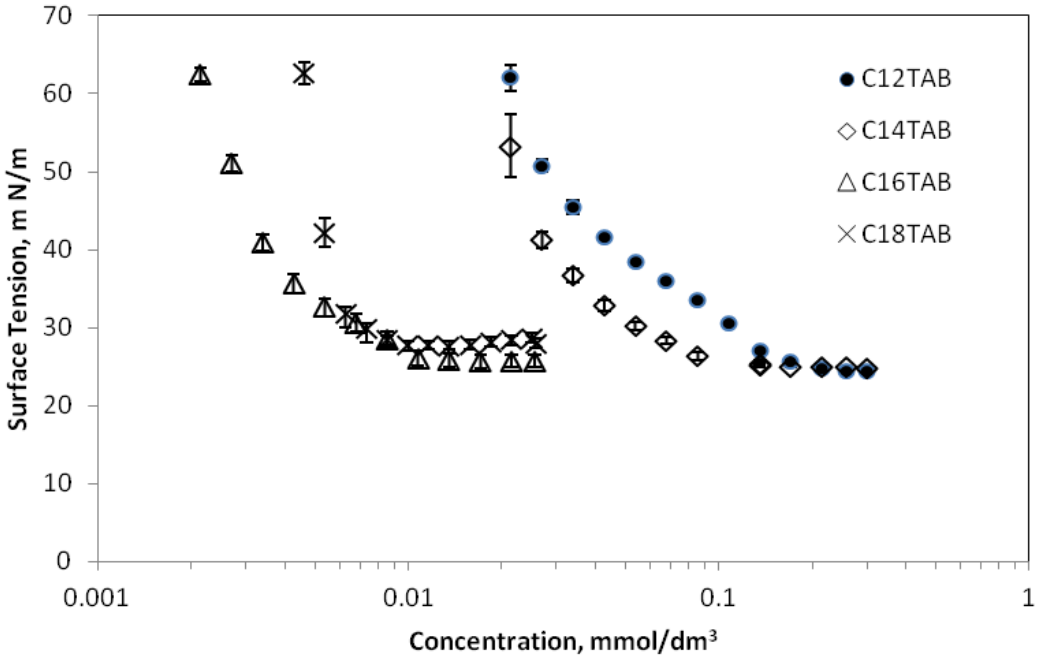


Figure 3.23 : Plots of surface tension against concentration for C_{12}MES.C_nTAB catanionic surfactants at 30˚C.

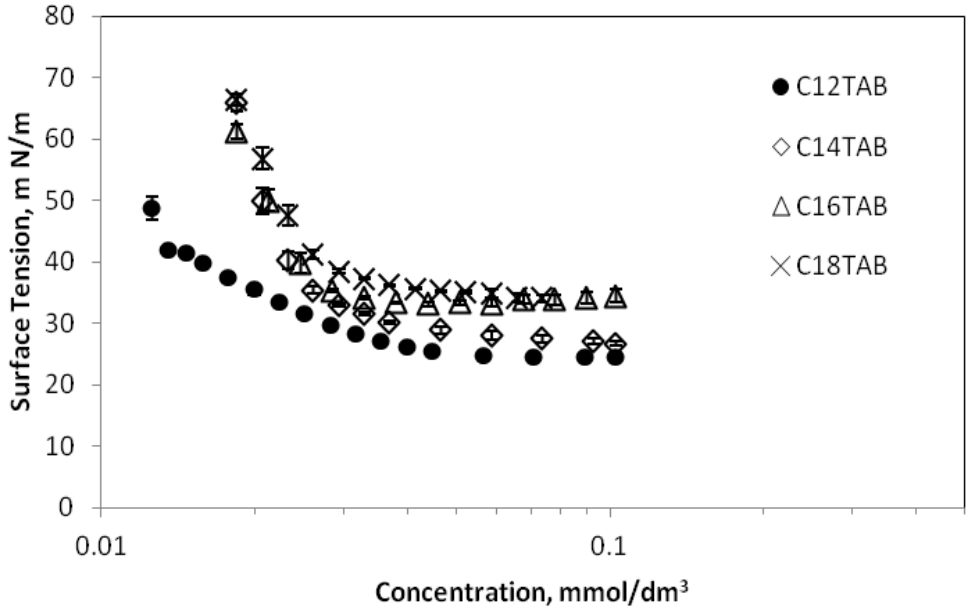


Figure 3.24 : Plots of surface tension against concentration for $C_{14}MES.C_nTAB$ catanionic surfactants at 30˚C.

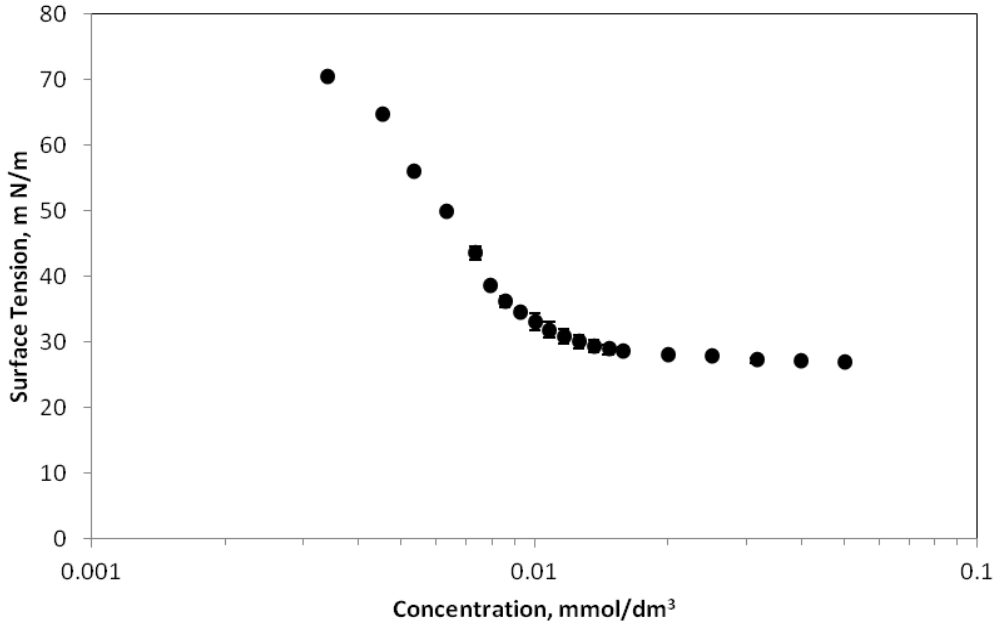


Figure 3.25 : Plots of surface tension against concentration for $C_{16}MES.C_{12}TAB$ catanionic surfactants at 30˚C.

Generally, increase in the alkyl chain length decreases the CMC values of the catanionic surfactants. This is due to the higher hydrophobicity of the surfactants that makes the hydrophobic tail held up straighter and thus occupying smaller area at the air/water interface. A similar trend was also observed in our previous work on anionic/cationic mixtures (Wong *et al*., 2012). Surface tension (ST) at CMC values were also tabulated in Table 3.8. Catanionic surfactants with lower alkyl chain length were found to have greater ability in decreasing the air/water interfacial tension.

The presence of salts in an aqueous surfactant system plays an important role in modifying both intermicellar and intramicellar interactions. Various studies have shown that inorganic salts can alter the charge screening effect between the surfactant molecules which consequently affect the phase behaviour (Fernandes *et al*., 2010; Hao *et al*., 2006). The difference between catanionic surfactants and anionic/cationic mixtures is that catanionic surfactants are salt free. In comparison with a previous work (Wong *et al*., 2012), CMC values of catanionic surfactants are lower than anionic/cationic mixed surfactants. Absence of NaBr salts reduces the repulsive interactions between surfactant molecules. Such phenomena favored closer packing of the surfactant molecules and thus enhanced the micellization process (Fernandes *et al*., 2010). This is also further confirmed by the surface excess concentration, Γ_{max} and minimum area, A_{min} values of the catanionic surfactants in Table 3.8. A_{min} values of $C_{12}MES.C_nTAB$ catanionic surfactants are in the range of 0.16 nm to 0.78 nm where else for $C_{12}MES.C_nTAB$ mixtures, A_{min} values are in the range of 0.42 nm to 1.70 nm (Wong *et al*., 2012). Similarly for $C_{14}MES.C_nTAB$ mixtures (Wong *et al*., 2012), A_{min} values are in the range of 0.27 nm to 1.70 nm as compared to A_{min} values of catanionic surfactants which are just between 0.13 nm and 0.58 nm. This showed that the

surfactant molecules of catanionic surfactants are in a more optimal packing condition as compared to its mixtures.

Table 3. 8 : Critical micelle concentration (CMC), surface tension at CMC, maximum surface excess (Γ_{max}) and minimum area (A_{min}) values of $C_mMES.C_nTAB$ catanionic surfactants.

C_mMES	**C_nTAB**	**CMC, mmol/dm^3**	**ST at CMC, m N/m**	**Γ_{max} x10^6, mol m^{-2}**	**A_{min}, nm^2**
12	12	0.1356	27.06	2.22	0.75
	14	0.0855	26.39	2.45	0.68
	16	0.0108	26.04	2.12	0.78
	18	0.0036	35.62	10.51	0.16
14	12	0.0448	25.42	2.87	0.58
	14	0.0293	33.00	8.49	0.20
	16	0.0285	35.34	10.70	0.16
	18	0.0247	44.44	12.91	0.13
16	12	0.0117	30.79	3.17	0.52
	14	-	-	-	
	16	-	-	-	
	18	-	-	-	

3.3 Biological Properties

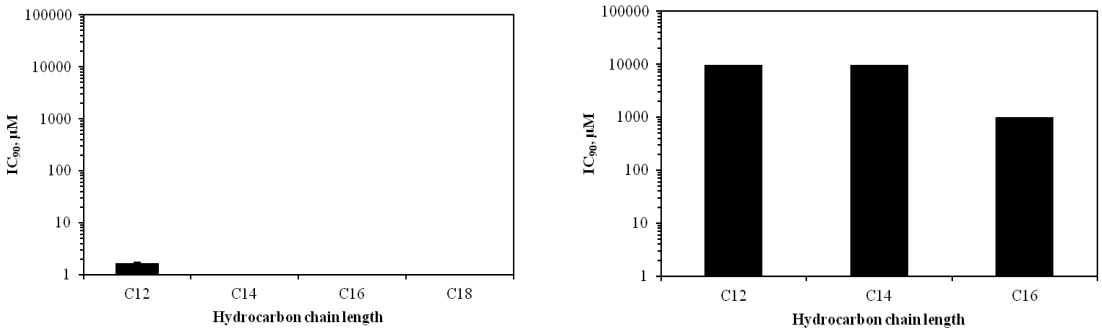
In the previous section, the surface properties of mixed alkyl trimethylammonium bromide (C_nTAB) and sodium methylester α-sulfoalkylate (C_mMES) surfactants systems were reported, where m denotes hydrocarbon chain length of 12 to 16 carbons and n demotes as 12 to 18 carbons long. While C_nTAB is a conventional cationic surfactant, C_mMES is relatively new anionic surfactant that has considerably good surface activity as compared to conventional anionic surfactants such as alkyl sulfate (AS) and linear alkylbenzene sulfonates (LAS). The C_mMES also exhibits better biodegradability than other anionic surfactants of its grade (Kapur *et al*., 1978; Stirton *et al*., 1961; Teruhisa, 1992). From the work, we found that C_mMES/C_nTAB mixed surfactants had lower tendency to form precipitates as compared to C_mLAS/C_nTAB mixed surfactants. Results from section 3.2 also showed that the mixed surfactants had better surface properties as compared to both of its single surfactants.

The aim of this work is to explore the antibacterial properties of (C_mMES/C_nTAB) catanionic surfactants and mixtures. Besides matching different hydrocarbon chain lengths, mixing ratios of the anionic/cationic mixtures were also varied. Potency of this C_mMES/C_nTAB surfactants was tested against common types of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria.

3.3.1 Anionic/Cationic Mixtures

3.3.1.1 Antibacterial Property of Single Surfactant System

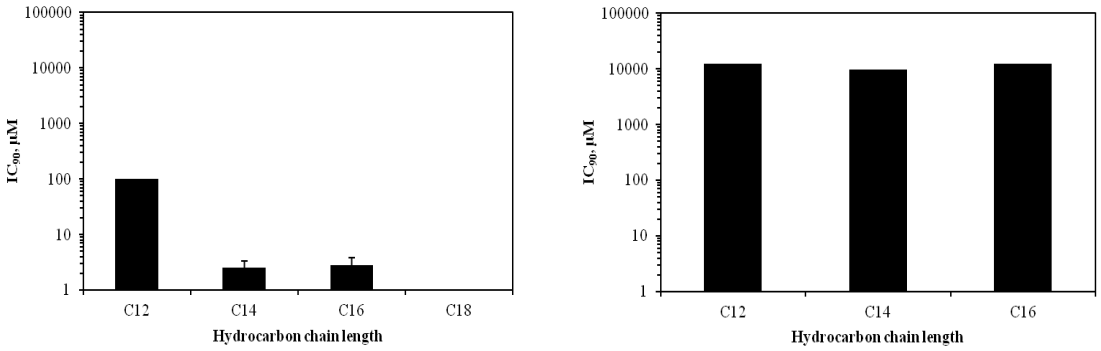
The effects of C_mMES and C_nTAB (where m = 12, 14 and 16, and n = 12, 14, 16 and 18) surfactant series against *S. aureus* are shown in Figure 3.24. Results indicated C_nTAB surfactant series were very active against *S. aureus*, with IC_{90} values lower than 2μM. The inhibitory effectiveness towards *S. aureus* increases with the increased of alkyl chain length of C_nTAB surfactants. Meanwhile C_mMES surfactants series exhibited IC_{90} values above 900 μM (which was more than 450 times more concentrated than C_nTAB surfactant series). Even though C_mMES surfactant series showed weak inhibitory activity, their trend on the increase of inhibitory effectiveness with increasing hydrophobic chain length towards *S. aureus* was similar to C_nTAB surfactant series.



(a) (b)

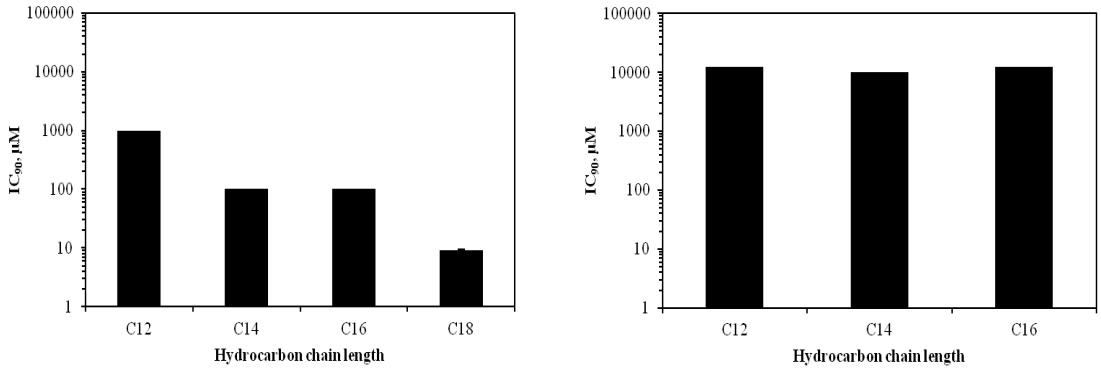
Figure 3.26 : Minimum inhibitory concentration, IC_{90} of (a) C_nTAB and (b) C_mMES surfactant series against S. *aureus*.

Inhibitory effectiveness of both C_mMES and C_nTAB surfactant series against two gram-negative bacteria (namely *E. coli* and *P. aeruginosa*) are shown in Figure 3.25 and 3.26. Both C_mMES and C_nTAB surfactant series were less effective against gram-negative bacteria as compared to the gram-positive bacteria, *S. aureus*. High concentration (above 9mM) of C_mMES surfactants (having different hydrocarbon chain length from m = 12 to 16) were needed to inhibit the growth of both gram-negative bacteria. C_nTAB surfactant series needs less than 100μM and even lesser concentration (below 5μM) as the hydrocarbon chain length increases from n = 12 to n = 18 when tested on *E. coli*. However, it was noted that higher concentrations (from 10 to 50 times more concentration) of C_nTAB surfactant (from n = 12 to n = 18) was needed to inhibit the growth of *P. aeruginosa* as compared to the former even though both were gram-negative bacteria.



(a) (b)

Figure 3.27 : Minimum inhibitory concentration, IC_{90} of (a) C_nTAB and (b) C_mMES surfactant series against E. *coli*.



(a) (b)

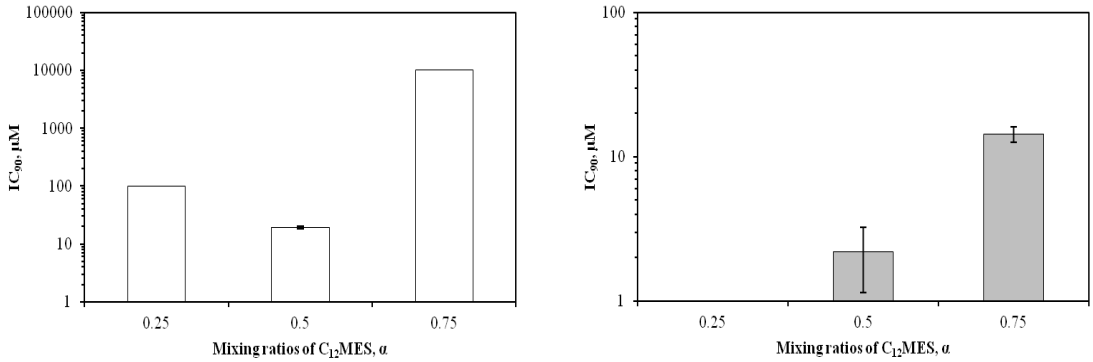
Figure 3.28 : Minimum inhibitory concentration, IC_{90} of (a) C_nTAB and (b) C_mMES surfactant series against P. *aeruginosa*.

3.3.1.2 Antibacterial Property of Anionic/Cationic Binary Surfactant System

In many instants, mixtures of more than one surfactant (either similar or different type) provide many advantages either in the physical or chemical properties over the use of a single surfactant. Individual surfactant series have been described in the earlier section. In this section, both anionic (C_mMES) and cationic (C_nTAB) surfactants series were mixed together into three different ratios (representing anionic-rich, equimolar and cationic-rich portions respectively) in the aqueous systems. Interesting inhibitory results exhibited by the anionic-cationic surfactant mixtures against the three types of microbes (*S. aureus*, *E. coli* and *P. aeruginosa*) were observed as indicated in Figure 3.27 – 3.35.

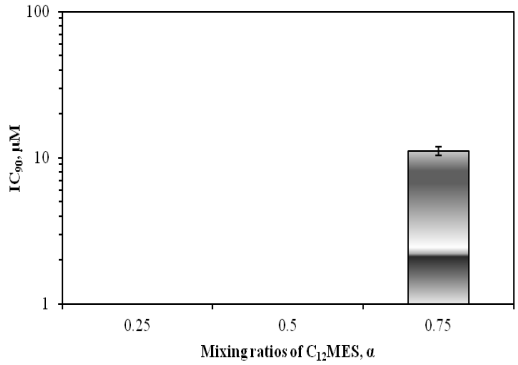
As indicated in Figure 3.27- 3.29, mixtures between C_mMES and C_nTAB surfactants series generally showed an increase in the inhibitory activity against *S. aureus* with the increase of hydrocarbon chain length (n = 12 to 18) in the hydrophobic portion of C_nTAB in all three C_mMES (where m = 12 to 16) molar ratios in mixed anionic-cationic surfactant systems except 0.5 molar ratio of mixed $C_{16}MES$ and $C_{18}TAB$ surfactant system. The weakening of inhibitory effectiveness was also noted with the increase of

hydrocarbon chain length in the hydrophobic portion of C_mMES (m = 12 to 16) respectively, except at anionic-rich molar ratio in the mixtures. Interestingly among all the mixed surfactant systems, both C_{12}MES/C_{12}TAB and C_{14}MES/C_{12}TAB mixed systems exhibited maximum inhibitory activity (20μM and 7μM, respectively) at equimolar ratio followed by cationic-rich and lastly anionic-rich ratio. It was believed that C_{16}MES/C_{12}TAB mixed systems also exhibited similar trend as the former even though IC_{90} values were lower than 1μM (below the tested concentration).

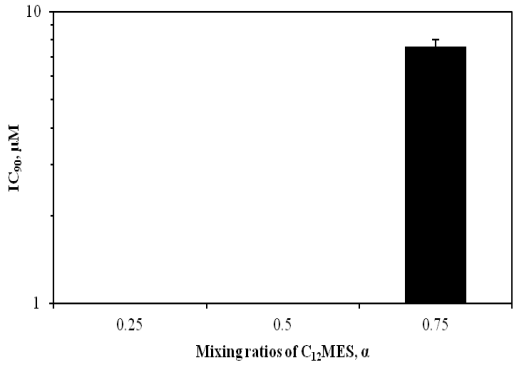


(a)

(b)



(c)



(d)

Figure 3.29 : Minimum inhibitory concentration, IC_{90} of (a) $C_{12}MES/C_{12}TAB$, (b) $C_{12}MES/C_{14}TAB$, (c) $C_{12}MES/C_{16}TAB$ and (d) $C_{12}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against S. *aureus*.

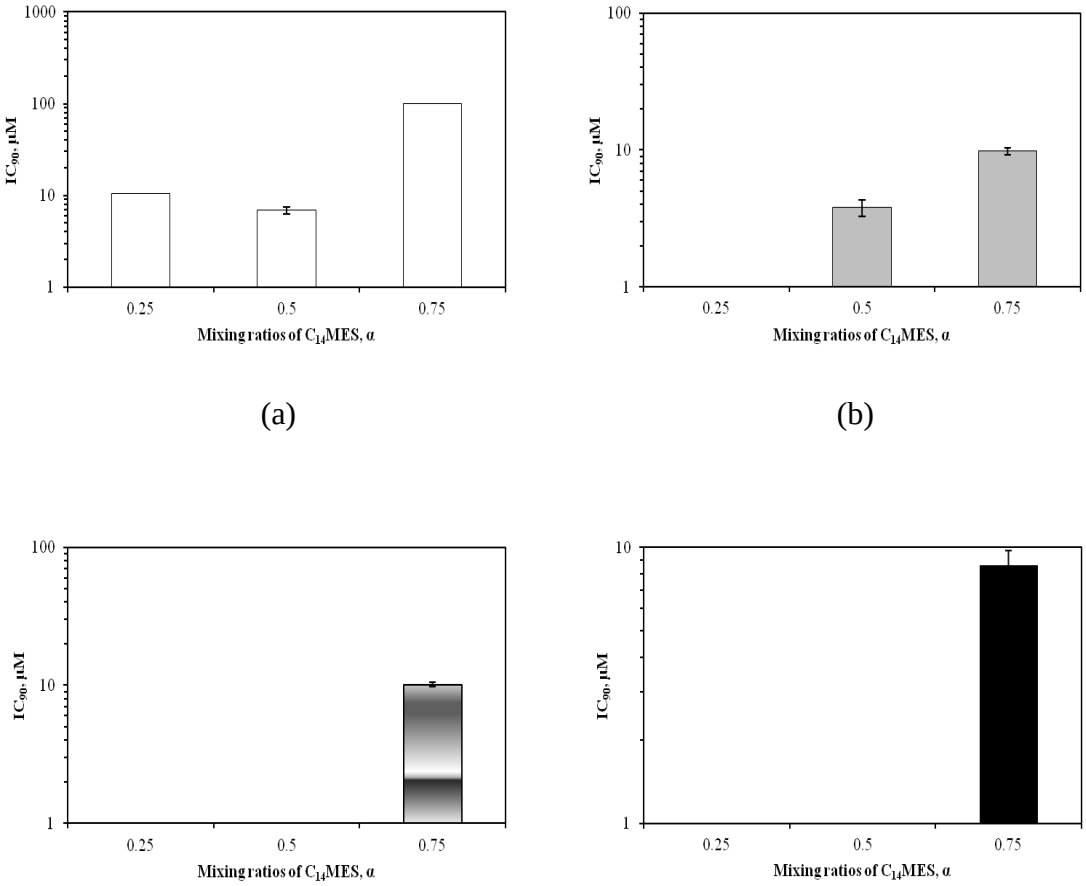


Figure 3.30 : Minimum inhibitory concentration, IC_{90} of (a) $C_{14}MES/C_{12}TAB$, (b) $C_{14}MES/C_{14}TAB$, (c) $C_{14}MES/C_{16}TAB$ and (d) $C_{14}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against S. *aureus*.

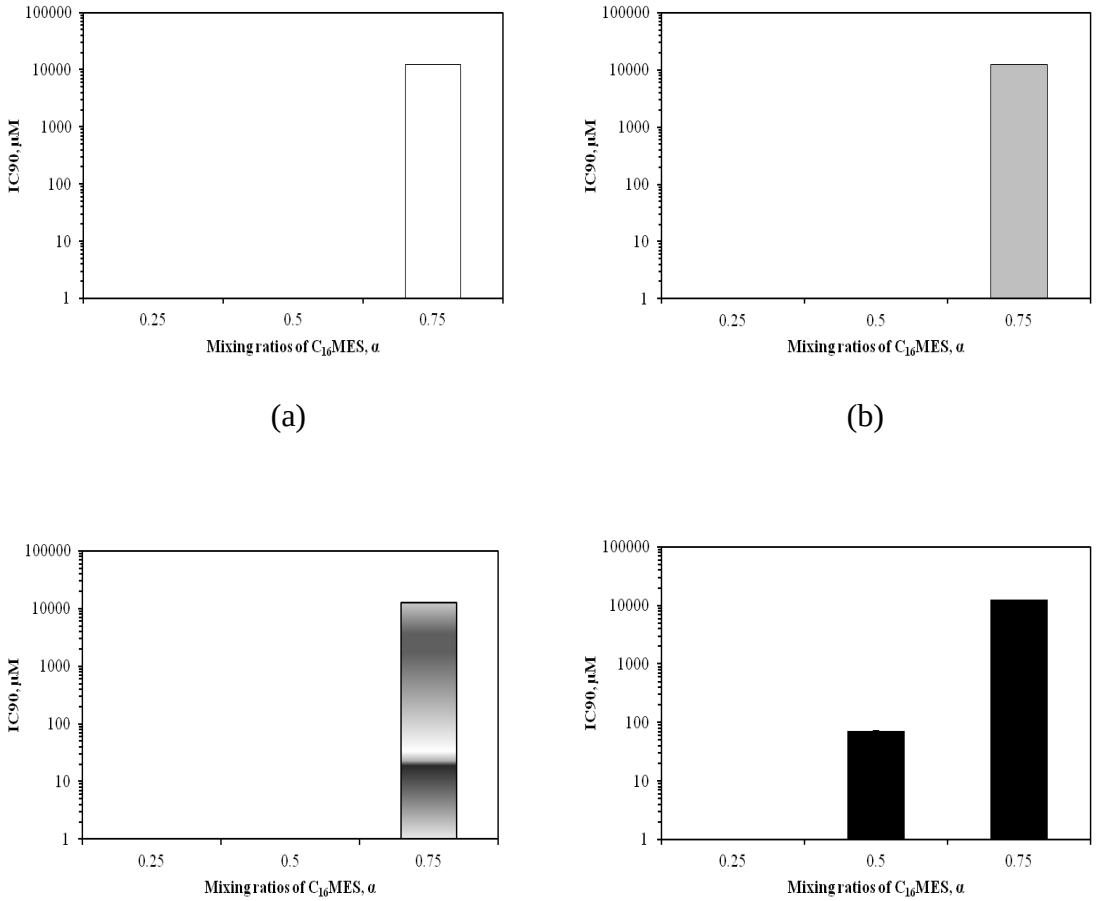


Figure 3.31 : Minimum inhibitory concentration, IC_{90} of (a) $C_{16}MES/C_{12}TAB$, (b) $C_{16}MES/C_{14}TAB$, (c) $C_{16}MES/C_{16}TAB$ and (d) $C_{16}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against S. *aureus*.

As noted in the previous section, gram-negative bacteria (both *E. coli* and *P. aeruginosa*) were quite resistant to C_mMES and C_nTAB surfactants series. However in general, similar trend as anionic-cationic surfactant mixtures against gram-positive bacteria were observed on the gram-negative bacteria where the inhibitory activity increases along with the increased amount of cationic surfactants in the mixed surfactant systems (except C_{12}MES/$C_{n'}$TAB, where n' = 12 - 16, exhibited reverse inhibitory activity trend and C_{16}MES/C_{18}TAB with no inhibitory change in the mixed systems) as shown in Figure 3.30 – 3.35. Another interesting finding was noted where optimum inhibition power was exhibited against *E. coli* on 0.5 and 0.75 molar ratios of C_{12}MES/C_{14}TAB and C_{12}MES/C_{16}TAB; and 0.25 and 0.5 molar ratios of C_{16}MES/C_{16}TAB mixed surfactants systems (see Figure 3.30 – 3.32). Similarly when tested on *P. aeruginosa*, where both 0.5 and 0.25 molar ratios of C_{12}MES/C_{16}TAB and C_{16}MES/C_{16}TAB shows optimum inhibitory concentration.

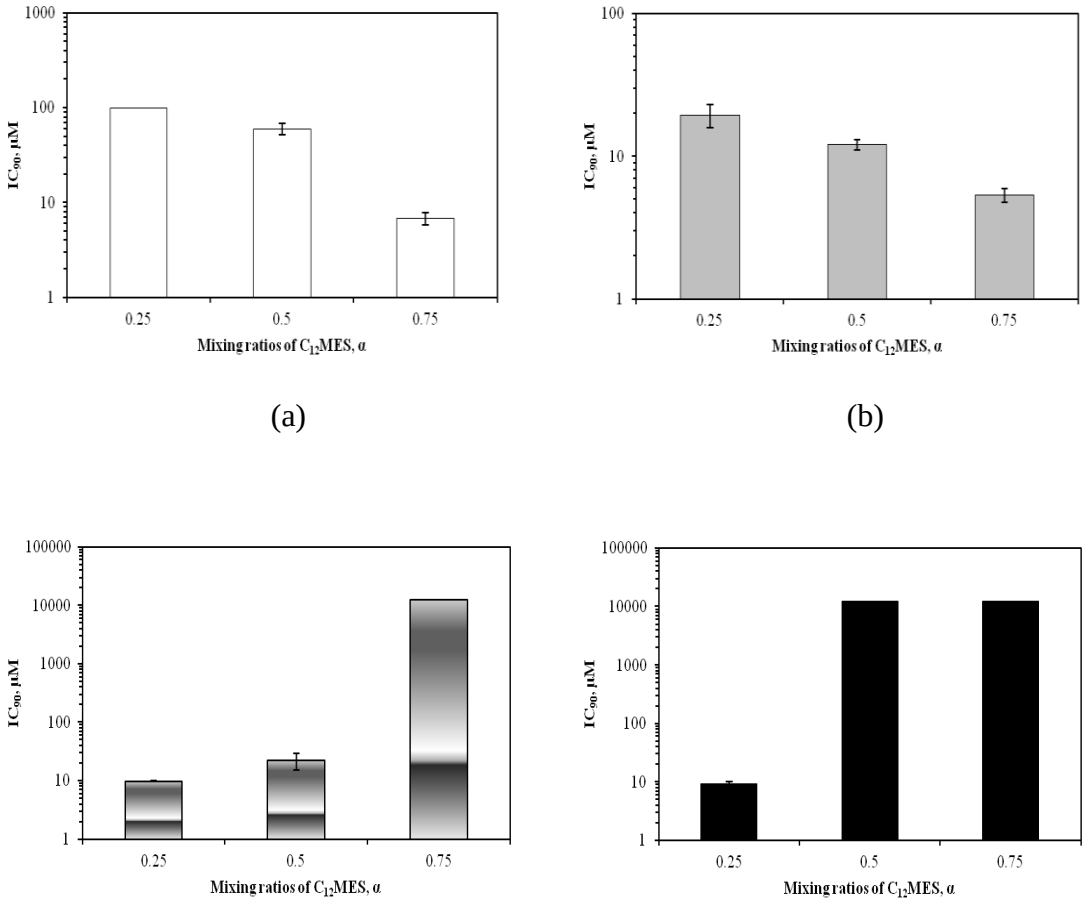


Figure 3.32 : Minimum inhibitory concentration, IC_{90} of (a) $C_{12}MES/C_{12}TAB$, (b) $C_{12}MES/C_{14}TAB$, (c) $C_{12}MES/C_{16}TAB$ and (d) $C_{12}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against E. *coli*.

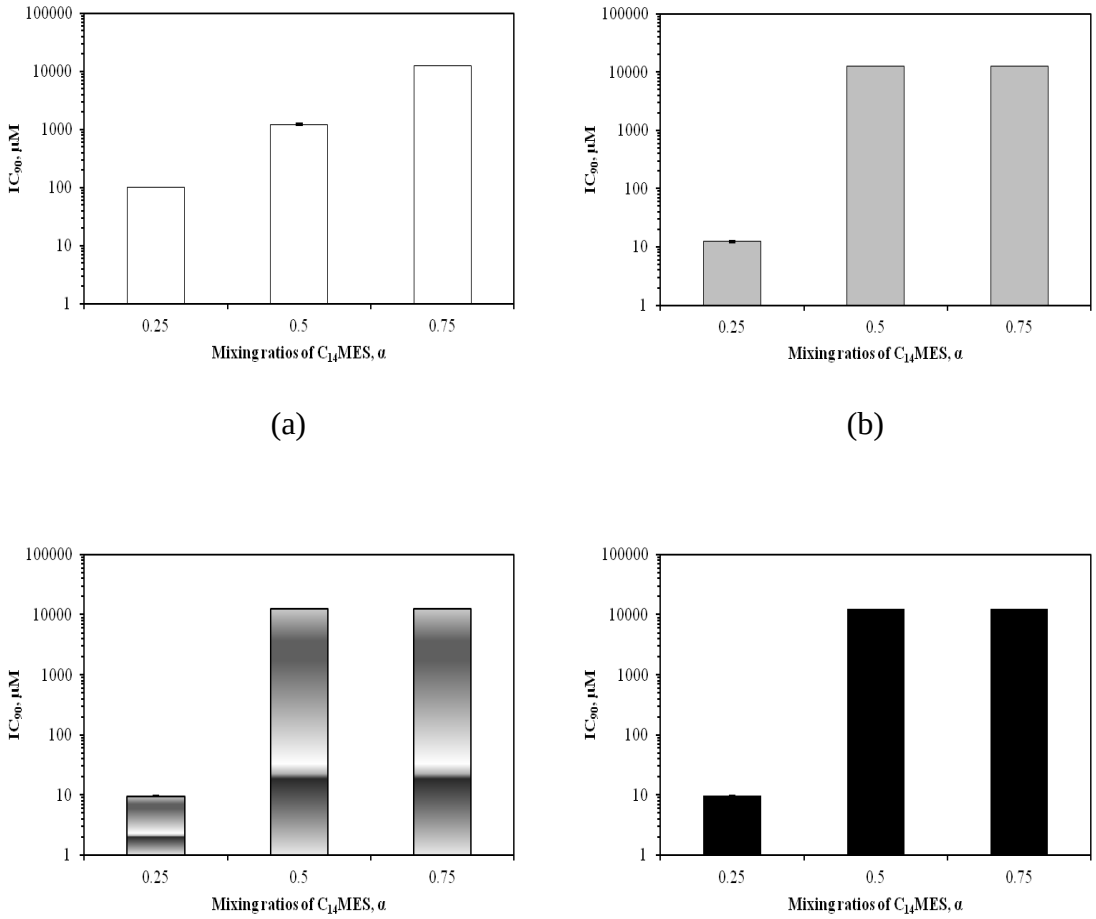


Figure 3.33 : Minimum inhibitory concentration, IC_{90} of (a) $C_{14}MES/C_{12}TAB$, (b) $C_{14}MES/C_{14}TAB$, (c) $C_{14}MES/C_{16}TAB$ and (d) $C_{14}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against E. *coli*.

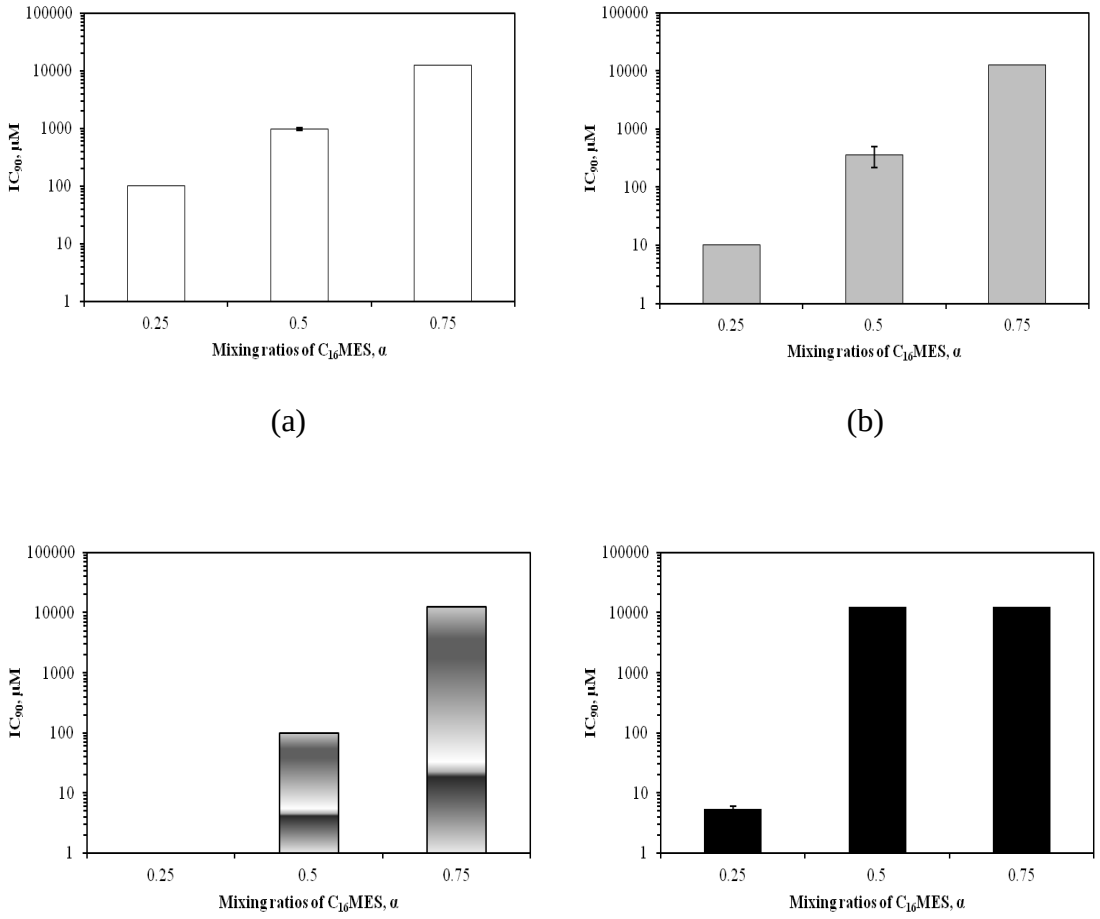


Figure 3.34 : Minimum inhibitory concentration, IC_{90} of (a) $C_{16}MES/C_{12}TAB$, (b) $C_{16}MES/C_{14}TAB$, (c) $C_{16}MES/C_{16}TAB$ and (d) $C_{16}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against E. *coli*.

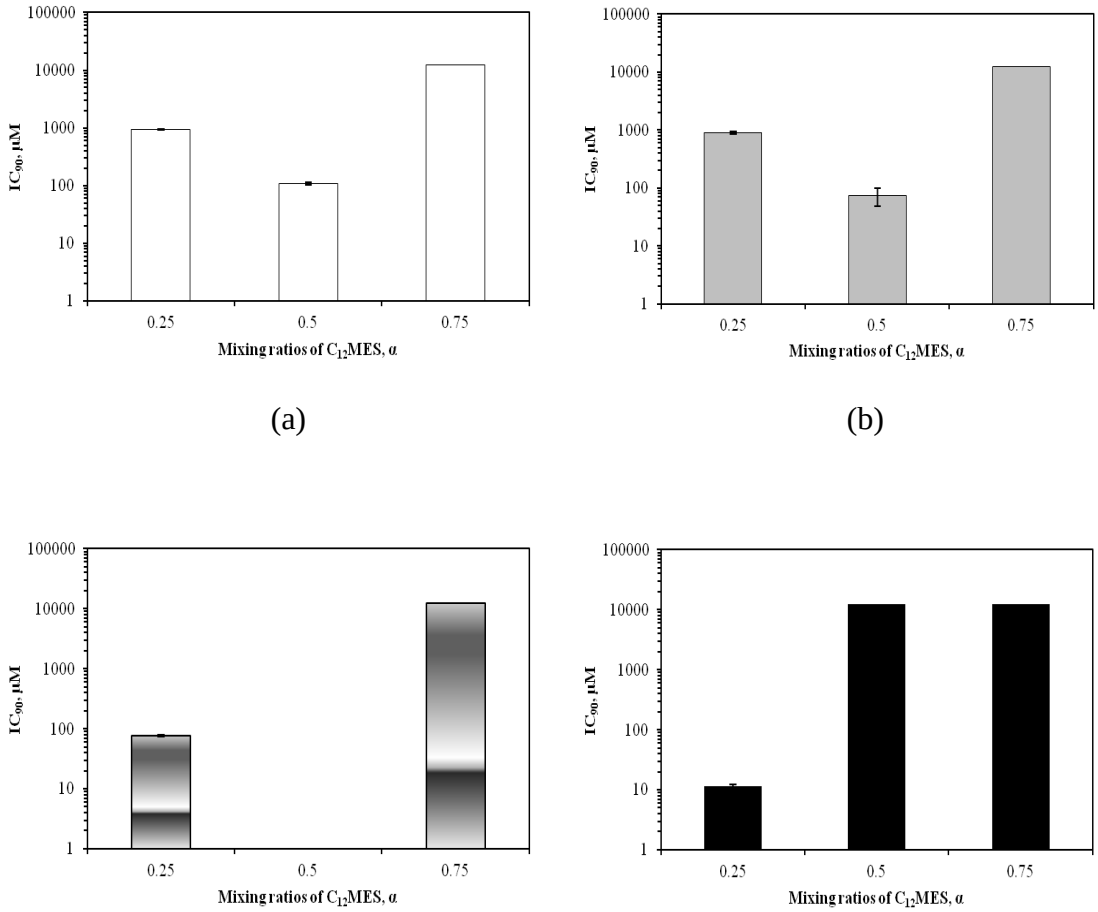


Figure 3.35 : Minimum inhibitory concentration, IC_{90} of (a) $C_{12}MES/C_{12}TAB$, (b) $C_{12}MES/C_{14}TAB$, (c) $C_{12}MES/C_{16}TAB$ and (d) $C_{12}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against P. *aeruginosa*.

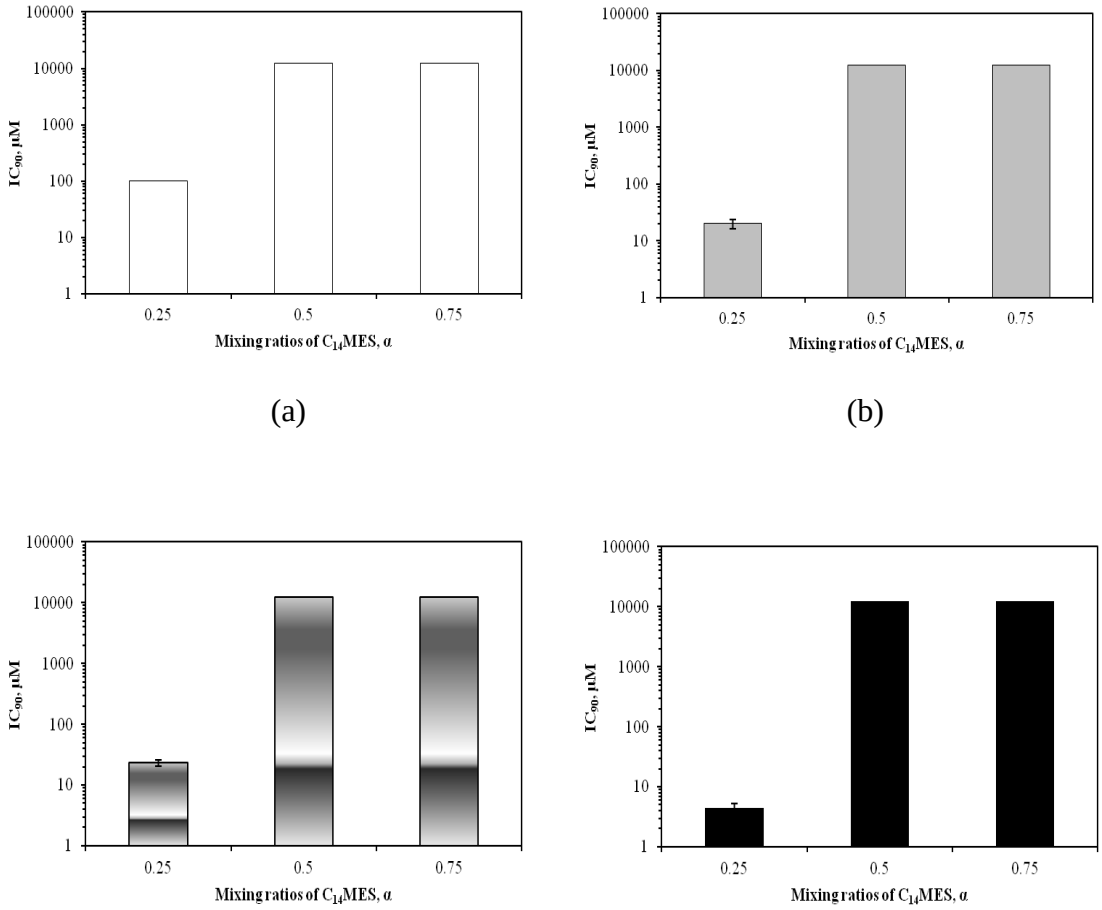


Figure 3.36 : Minimum inhibitory concentration, IC_{90} of (a) $C_{14}MES/C_{12}TAB$, (b) $C_{14}MES/C_{14}TAB$, (c) $C_{14}MES/C_{16}TAB$ and (d) $C_{14}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against P. *aeruginosa*.

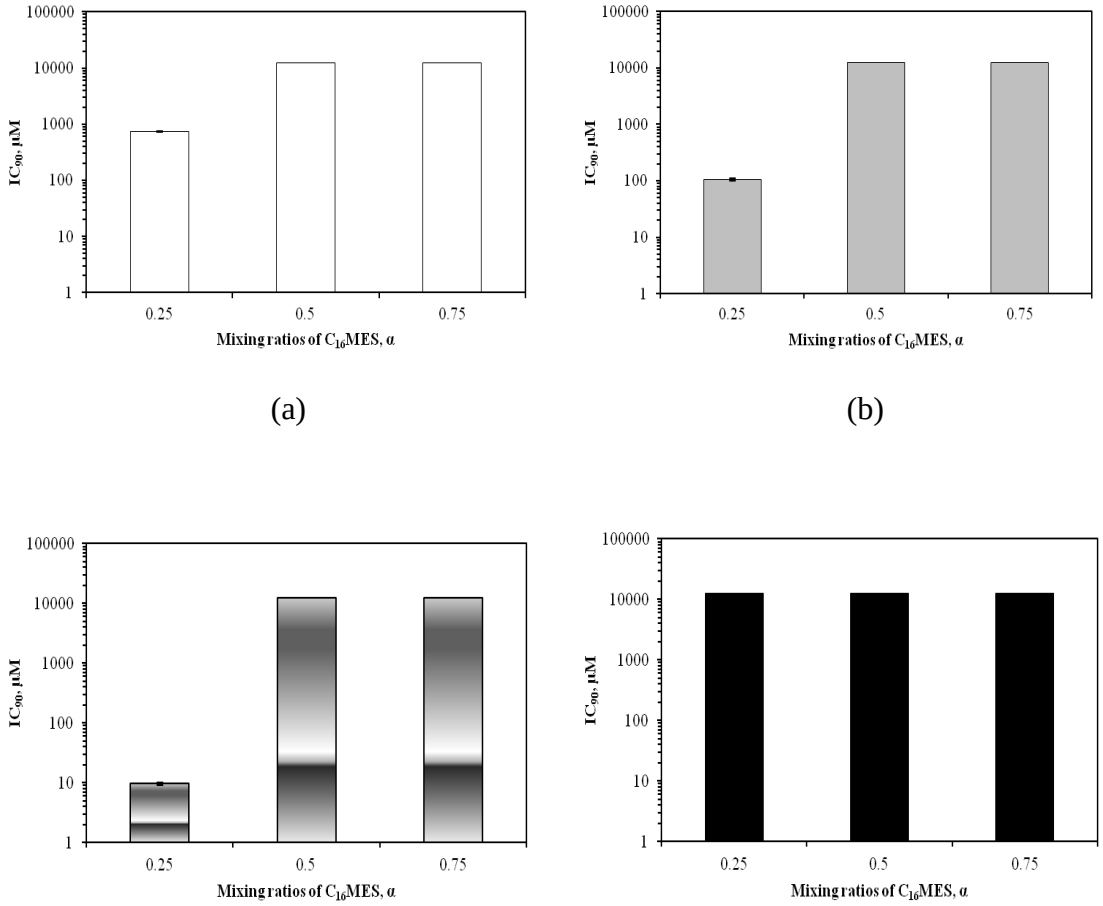


Figure 3.37 : Minimum inhibitory concentration, IC_{90} of (a) $C_{16}MES/C_{12}TAB$, (b) $C_{16}MES/C_{14}TAB$, (c) $C_{16}MES/C_{16}TAB$ and (d) $C_{16}MES/C_{18}TAB$ surfactant mixtures at different molar ratios against P. *aeruginosa*.

In general *S. aureus* is more susceptible to antibacterial agents as compared to *E. coli* and *P. aeruginosa.* This was because *S. aureus* (gram-positive bacteria) does not have an outer membrane which acts as a barrier against antibacterial agent (Russell *et al*., 1982). Whereas gram-negative bacteria (such as E. *coli* and *P. aeruginosa*), contain an outer membrane wall (consist of lipopolysaccharide and protein) which envelope the bacteria. On top of that, *P. aeruginosa* is able to produce slime which it covers itself in, thus, it is exceptionally resistant to chemical agents (Brown, 1971; Stickler, 1982). Previous studies of QAC compounds against these bacteria have indicated that the order of increase resistivity were as followed where S. *aureus* < E. *coli* < *P. aeruginosa* (Russell *et al*., 1982; Hamilton, 1971).

Surfactant molecules, which have both polar and non-polar portions, exhibited the ability to interact with the lipid layer of cell membranes wall of the organism. This interaction may result in changing the membrane orientation which could solubilise, damage and destruct the membrane that lead to the death of the cell. Results in Figure 3.24 – 3.35 showed that the biological activities of the tested surfactant (both individual and anionic-cationic mixtures) compounds were depended on both the character of polar head (size and electrical charge distribution) and hydrophobic hydrocarbon chain length. The length of the alkyl chain length of the surfactant substances incorporated into the membranes affects the biological activity. This was clearly demonstrated by the increased of hydrophobic alkyl chain length from dodecyl to octadecyl chain of cationic surfactants, C_nTAB, increases the biological activity against all tested bacteria (S. *aureus*, E. *coli* and *P. aeruginosa*). This was attributed to the increase in adsorption tendency of C_nTAB surfactants onto the bacteria membrane surface (which disrupts the membrane of the bacteria) with the increase hydrophobic chain length as indicated in their surface activity properties. Long alkyl chain length of the hydrophobic portion

increases the adsorption of the molecules at the interfaces. On the contrary, surfactants having shorter hydrophobic chains will have a lower tendency towards adsorption at the interfaces. Apart from these, MIC values for all C_nTAB surfactants occurred below the critical micelle concentrations (CMC) values. This showed that the surfactant monomers are the species that play the crucial role in interacting with the membrane cells and not the aggregates (Morán *et al*., 2001).

Similar to C_nTAB surfactant series, the increases of hydrophobic chain length of C_mMES series, increases the inhibitive power towards gram-positive bacteria, S. *aureus*. However, a normal bell-shaped curve with a maximum inhibitive power at 14 carbon atoms in the alkyl chain length of the hydrophobic group was obtained against gram-negative bacteria, E. *coli* and *P. aeruginosa*,. Such an inhibitive trend (optimum biological effects at a chain length of 14 carbon atoms) have also been observed in other studies (Bartnik, 1992; Balgavý and Devínsky, 1996). This phenomenon was attributed to a combination of physico-chemical properties of the surfactants (i.e. CMC, absorption, aqueous solubility and hydrophobicity) (Morán *et al*., 2001; Balgavý and Devínsky, 1996). Besides this, the morphology of the biological bilayer membrane (i.e bilayer thickness, stability and hydrophobicity) also plays a crucial role in affecting the inhibitive mechanism of surfactants (Balgavý and Devínsky, 1996). As noted in Figure 3.24 – 3.26, anionic surfactants are not a good antibacterial agent on its own as a high concentration was needed to inhibit the growth of bacteria, especially against gram-negative bacteria (Bartnik, 1992).

In the mixed C_mMES/C_nTAB surfactant systems (as indicated in Figure 3.27-3.35), synergistic inhibitive activity effect against bacteria growth (on both gram-positive and gram-negative types) were noted. The inhibitory potency differences between the

individual (of both C_mMES and C_nTAB) surfactant and C_mMES/C_nTAB mixtures on the bacteria growth can be explained through their differences in polar head (size and electrical charge distribution) and hydrocarbon chain length.

Basically, the bacterial inhibition efficacy of C_mMES/C_nTAB mixtures is due to the presence of the positively charged nitrogen atoms (Nabel *et al*., 2010). Therefore, the MIC values of the mixtures were more affected by the change of alkyl chain length of the C_nTAB surfactants instead of C_mMES. Though mixed surfactants at the cationic-rich region were expected to have the highest inhibitive power among the different mixing ratios, results from Figure 3.27-3.35 showed otherwise, where mixtures at an equimolar mixing ratio have the highest potency. This could be explained by the close packing of the mixed surfactant molecules at this mixing ratio (Wong *et al*., 2012). Usually, close packing of surfactant molecules were associated with high adsorption tendency at the interface. Hence, the inhibitive power of the C_mMES/C_nTAB mixtures was also raised.

However, a reverse inhibitory activity (where the inhibition strength decreases from anionic-rich to equimolar and lastly cationic-rich ratio) for C_{12}MES/C_nTAB mixed systems was also observed. This could be due to the size of the aggregations formed were smaller in comparison to cationic-rich and equimolar mixing ratio of the mixed systems. Smaller aggregations allowed the actives to penetrate easily through the cellular membrane and thus disrupt the membrane. Besides that, adsorption of the aggregates increases with desreasing size (Vieira and Carmona-Ribeiro, 2006).

Besides this, hydrophobicity of the mixed surfactants also played a crucial role in the inhibitive efficacy against the bacteria tested. As the hydrocarbon chain length of C_mMES or C_nTAB surfactant increases, the surface activity of the mixtures also

increases. This trend was shown in our previous paper (Wong *et al*., 2012). High surface activity means that the surfactant molecules have a higher tendency to adsorb at the interface and therefore more readily to destruct the membrane of the bacteria (Nabel *et al*., 2010).

Despite the long alkyl chain length of C_{14}MES/C_{18}TAB and C_{16}MES/C_{18}TAB mixtures, their inhibitive power towards E. *coli* and *P. aeruginosa* were relatively low or even exhibited no activity. This can be explained by the compatibility of the surfactant molecules length with the membrane thickness. Partitioning of the surfactant into the membrane were favored when the length of the surfactant molecules were similar with the thickness of the membrane. Meanwhile, surfactant molecules that are too big or long would have difficulties to diffuse into the membrane layer. Therefore, its inhibitive power was greatly reduced (Balgavý and Devínsky, 1996). On top of this, limited aqueous solubility of the long chain length surfactant mixtures could also be one of the factors that cause the decrease of inhibitive ability (Balgavý and Devínsky, 1996).

Addition of anionic surfactants, C_nMES did not directly inactivate the inhibitive activity of QAC in cationic surfactants, C_nTAB. For *P. aeruginosa*, certain systems even can enhance the inhibitive power of the cationic surfactants. In view of the toxicity against bacteria, not only the different alkyl chain lengths of the surfactants, but also the different mixing ratios play a crucial role.

3.3.2 Catanionic Surfactants

3.3.2.1 Antibacterial Property of Catanionic Surfactants

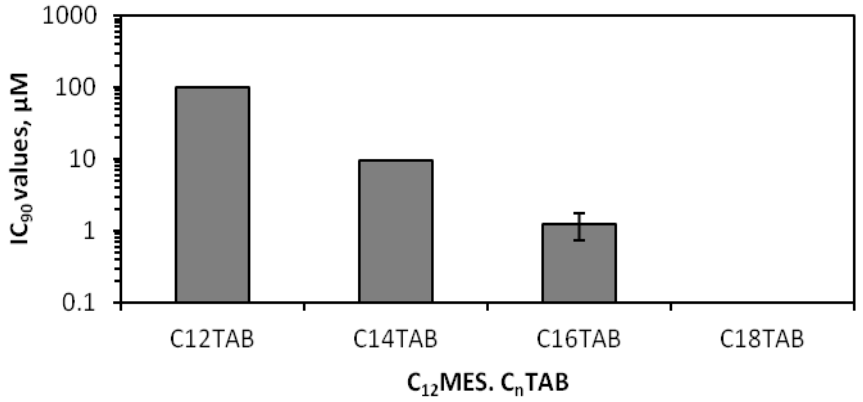
Inhibitory activities of catanionic surfactants against *S. aureus* are presented in Figure 3.36. Results showed that catanionic surfactants were very active against *S. aureus* with IC^{90} values as low as 1.25 μM. It was also observed that catanionic series generally showed increased inhibitory activity against *S. aureus* with increasing of hydrocarbon chain length of C_nTAB portion. This was mainly due to the raise in surface activity of catanionic surfactants as the hydrophobicity portion increases. This enhanced the ability of catanionic molecules to adsorb and partition into the bacteria membrane (McDonnell and Russell, 1999). However, inhibitory ability of catanionic series was reduced by 10 times with the increase of hydrocarbon chain length of C_mMES. As mentioned in the previous section, inhibitive activity of catanionic surfactant was attributed by the positively charged nitrogen atoms (Nabel *et al.,* 2010). Increase in C_mMES alkyl chain length will decrease the ratio of nitrogen atom in the surfactant molecules. Besides that, free volume created on the membrane bilayer of bacteria by partitioning of surfactants also contributes to lysis (Balgavý and Devínsky, 1996). As the C_mMES alkyl chain length increases, it closes the gap of the free volume created by the mismatch in hydrocarbon chain length of C_nTAB and C_mMES. Thus, catanionic surfactants with longer alkyl chain length of C_mMES are less potent to the growth of bacteria.

Inhibitory properties of catanionic surfactants against E. *coli* and *P. aeruginosa* are shown in Figure 3.37 and Figure 3.38 respectively. Results showed that catanionic surfactants were weak in inhibiting the growth of Gram-negative bacteria. This was due to the quaternary ammonium compound itself is not a very strong antibacterial agent for Gram-negative bacteria (Russell and Hugo, 1982; Hamilton, 1971; Morán *et al.,* 2001). Catanionic series with longer hydrocarbon chain length showed no activity towards both

bacteria strands. This is due to the cut-off effect in inhibition power of a particular killing agent where its ability is governed by solubility, interfacial properties, aggregate size, compatibility of the surfactant molecules length with the membrane thickness and etc. (Chernyavskaya *et al.*, 1984; Hamilton, 1971).

Overall, catanionic surfactants are less potent towards all three microbes as compared to its anionic/cationic mixtures (Wong *et al.*, 2012b). This could be due to the different aggregation behaviour of the two systems. Almost all of the CMC values of catanionic surfactants were lower than the IC^{90} values. This showed that the inhibition activity happens beyond micellization process. As described in previous work (Wong *et al.*, 2012b), inhibitive activity was induced by the penetration of surfactant molecules into the membrane of the bacteria and causes the membrane to lyse (Salton, 1968; McDonnell and Russell, 1999). In the case of catanionic surfactants, most of the surfactant molecules are assembled in micelles or vesicles aggregation with little free surfactant molecules in the bulk system. This consequently lowers the kinetics of the surfactant molecules to adsorb and partition into the bacteria membrane. Besides that, the bigger aggregation size of catanionic vesicles also reduces its inhibitive power as penetration into the membrane cell will be more difficult (Vieira *et al.*, 2006).

Additionally, the reason being for longer alkyl chain length catanionic surfactants to have low or none inhibitive activity could be due to the high ST value at the CMC as shown in Table 3.8. This characteristic shows that the catanionic surfactants have lower ability in affecting the interfacial tension. Thus leads to lower ability in partitioning into the bacteria membrane.



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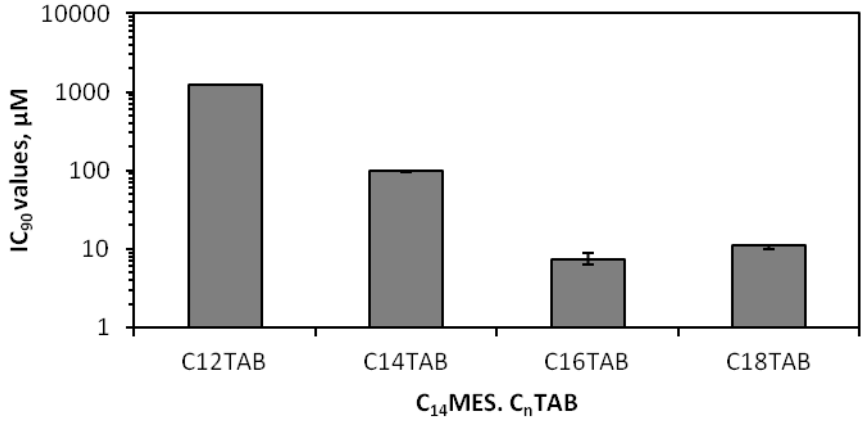

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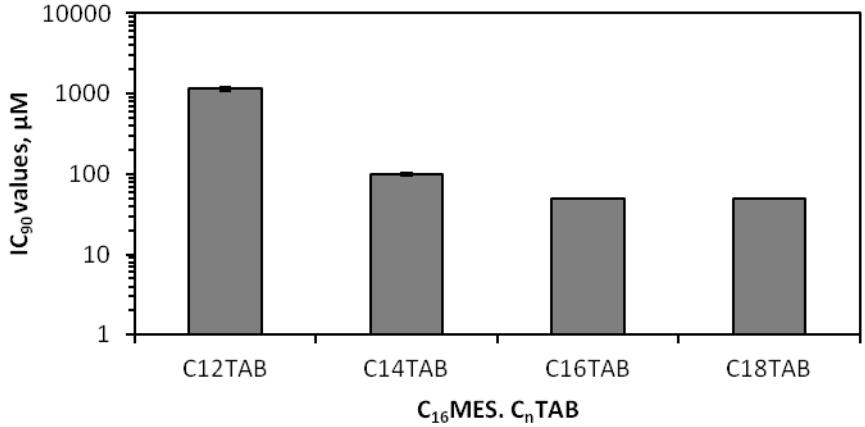
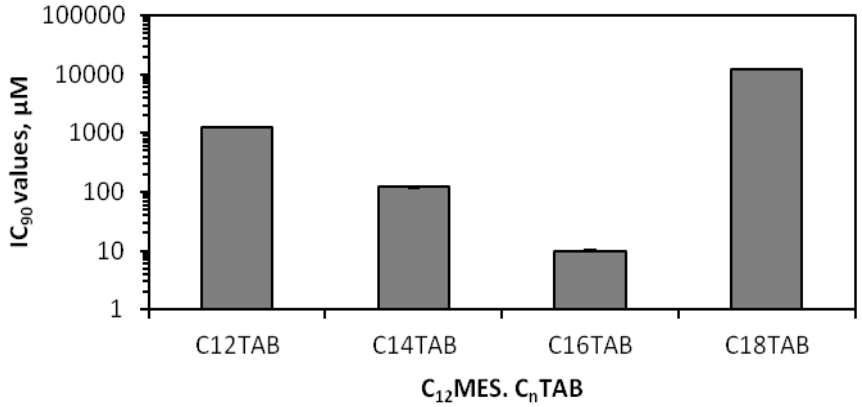


(c)

Figure 3.38 : Minimum inhibitory concentration, IC_{90} of (a) $C_{12}MES.C_nTAB$, (b) $C_{14}MES.C_nTAB$ and (c) $C_{16}MES.C_nTAB$ catanionic surfactant against S. *aureus*.



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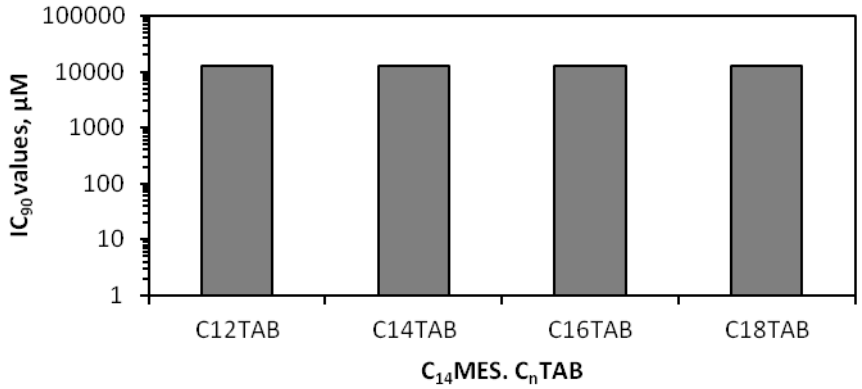

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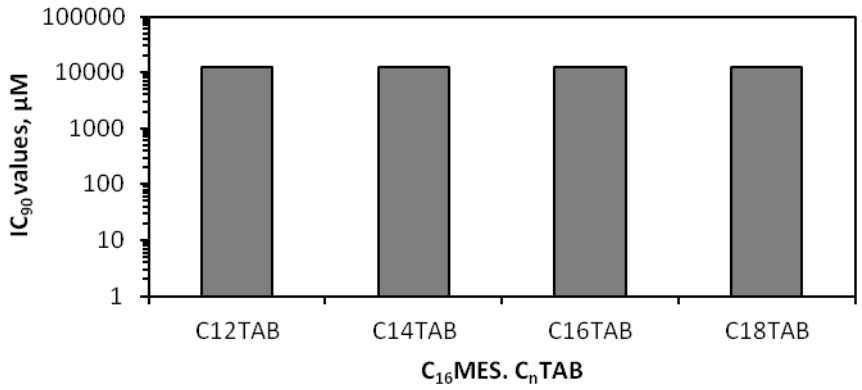
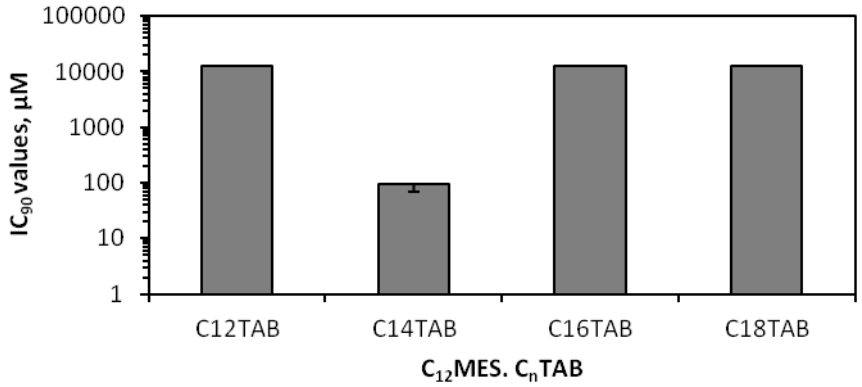


(c)

Figure 3.39 : Minimum inhibitory concentration, IC_{90} of (a) $C_{12}MES.C_nTAB$, (b) $C_{14}MES.C_nTAB$ and (c) $C_{16}MES.C_nTAB$ catanionic surfactant against E. *coli*.



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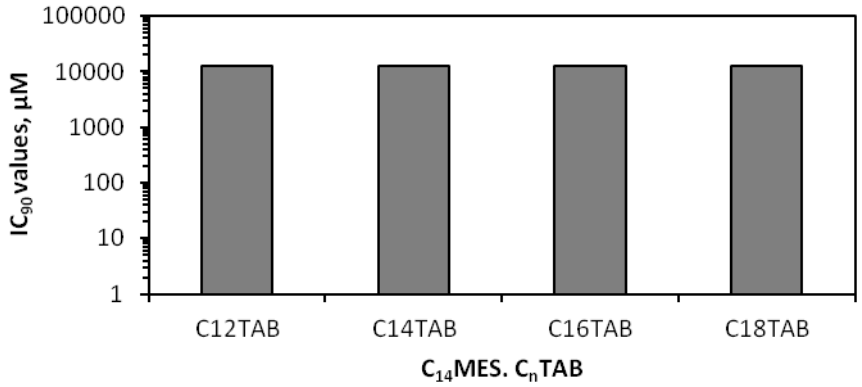
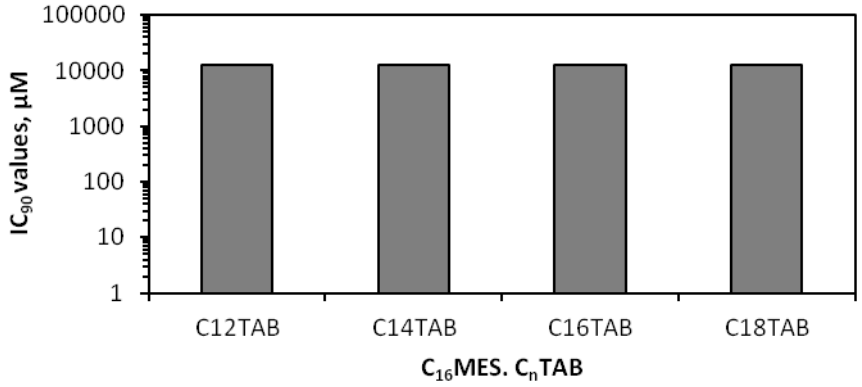


(b)



(c)

Figure 3.40 : Minimum inhibitory concentration, IC_{90} of (a) $C_{12}MES.C_nTAB$, (b) $C_{14}MES.C_nTAB$ and (c) $C_{16}MES.C_nTAB$ catanionic surfactant against P. *aeruginosa*.

CHAPTER FOUR

CONCLUSION

All $C_mMES.C_nTAB$ catanionic surfactants were successfully formed *via* the precipitation method which was modified to include a cold filtration system. Only $C_{12}MES.C_{18}TAB$, $C_{14}MES.C_{12}TAB$ and $C_{16}MES.C_{12}TAB$ catanionic systems needed recrystallization with acetone to obtain larger crystals for filtration. All $C_mMES.C_nTAB$ catanionic surfactants are white solids in appearance. Spectroscopic characterization from ^{1}H NMR, ^{13}C NMR and FTIR showed that the $C_mMES.C_nTAB$ catanionic surfactants inherent functional groups are from both of its parent surfactants. Spontaneous formation of vesicles was also observed under optical polarizing microscope when $C_mMES.C_nTAB$ catanionic surfactants were dispersed in water at a concentration of 1.0 $mgmL^{-1}$.

The surface activities of anionic/cationic mixtures were investigated using a surface tensiometer via du Nuoy ring method. The CMC values of all the anionic/cationic mixtures were less than values of CMC* and CMC of single surfactants, indicating strong synergistic interactions among the surfactant molecules in the mixed micelle formation. The interaction parameters of the mixed surfactants at liquid/air interface monolayer, β_{mon} and in the micelle, β_{mic} were highly negative that supported the existence of strong synergistic interactions between the surfactant molecules. Both β_{mic} and ΔG°_{ex} values agreed that $C_{12}MES/ C_{18}TAB$, $C_{14}MES/C_{14}TAB$ and $C_{16}MES/C_{12}TAB$ systems achieved optimum packing in the mixed micelles in their respective C_mMES series. The presence of oppositely charged surfactants at the interface reduced repulsion and the minimum area per molecule decreased. Thus, higher adsorption effectiveness of

mixed surfactants was achieved. It was also found that the effect of hydrocarbon chain length of cationic surfactants were more dominant as compared to anionic surfactants. In comparison to other anionic/cationic mixtures, C_mMES/C_nTAB mixed systems exhibit intermediate surface properties performance in which interactions at the surfactant head groups were found to be the dominant role.

Surface activities of C_mMES.C_nTAB catanionic surfactants were also investigated using a tensiometer via du Nuoy ring method except for C_{16}MES.C_{14}TAB, C_{16}MES.C_{16}TAB and C_{16}MES.C_{18}TAB systems which are not soluble at 30 ℃. CMC values of C_mMES.C_nTAB catanionic surfactants were lower than anionic/cationic mixed surfactants. The absence of NaBr salts reduces the repulsive interactions between surfactant molecules and allows closer packing between the molecules. This is further confirmed by the surface excess concentration, Γ_{max} and minimum area, A_{min} values of the catanionic surfactants. Besides that, catanionic surfactants with lower alkyl chain length were found to have a greater ability in decreasing the air/water interfacial tension.

Biological properties of anionic/cationic mixed surfactants and C_mMES.C_nTAB catanionic surfactants were studied via minimum inhibitory concentration (MIC) assay employing the broth dilution method. Both anionic/cationic mixed surfactants and C_mMES.C_nTAB catanionic surfactants exhibited good inhibition power towards Gram-positive bacteria, SA but weaker inhibition power towards Gram-negative bacteria, EC and PA. Inhibitive power of anionic/cationic mixtures was on par with single C_nTAB surfactants; with certain mixtures even have lower minimum inhibition concentration (MIC) than single C_nTAB surfactants. In addition, anionic/cationic mixed surfactants at equimolar mixing ratio showed exceptional inhibitive power against SA and EC. Although catanionic surfactants have higher surface activities, but they were less potent

towards all three microbes as compared to its anionic/cationic mixtures. This is due to the difference in killing mechanism towards the bacterial cells.

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LIST OF PUBLICATIONS

1. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2011). Physico-chemical Properties of Mixed Anionic/Cationic Surfactant Solution: Mixtures of Sodium Laurate Methyl Ester α-Sulfonate and Tetradecyltrimethylammonium Bromide. *Journal of Oil Palm Research* **23**, April 2011, p968-973. [ISI- Q4, 0.148].

2. Wong S P, Lim W H, Cheng S F and Chuah C H. (2012). Biological properties of sodium alkyl methyl ester sulfonate-alkyltrimethylammonium bromide surfactant mixtures. *Colloids and Surfaces B: Biointerfaces* **89**, January 2012, p48-52. [ISI- Q2, 2.78].

3. Wong S P, Lim W H, Cheng S F and Chuah C H. Properties of Sodium Methyl Ester Alpha-Sulfo Alkylate/Trimethylammonium Bromide Mixtures. *Journal of Surfactants and Detergents* **15**, March 2012, p601-611. [ISI- Q3, 1.239].

4. Wong S P, Lim W H, Cheng S F and Chuah C H. (2014). Synthesis, surface activities and antibacterial properties of novel alkyltrimethylammonium-methyl ester alpha-sulfo alkylate catanionic surfactants. *Journal of Oleo Science* [Submitted].

LIST OF PAPERS PRESENTED

1. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2012). Biological properties of catanionic surfactants. Poster presented at Oils and Fats International Congress 2012 (OFIC), 12 – 14 September 2012, Kuala Lumpur Convention Centre, Kuala Lumpur.

2. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2011). Antibacterial activity of mixed anionic-cationic surfactant solution. Poster presented at International Palm Oil Congress (PIPOC), 14 Nov to 17 Nov 2011, Kuala Lumpur Convention Centre, Kuala Lumpur.

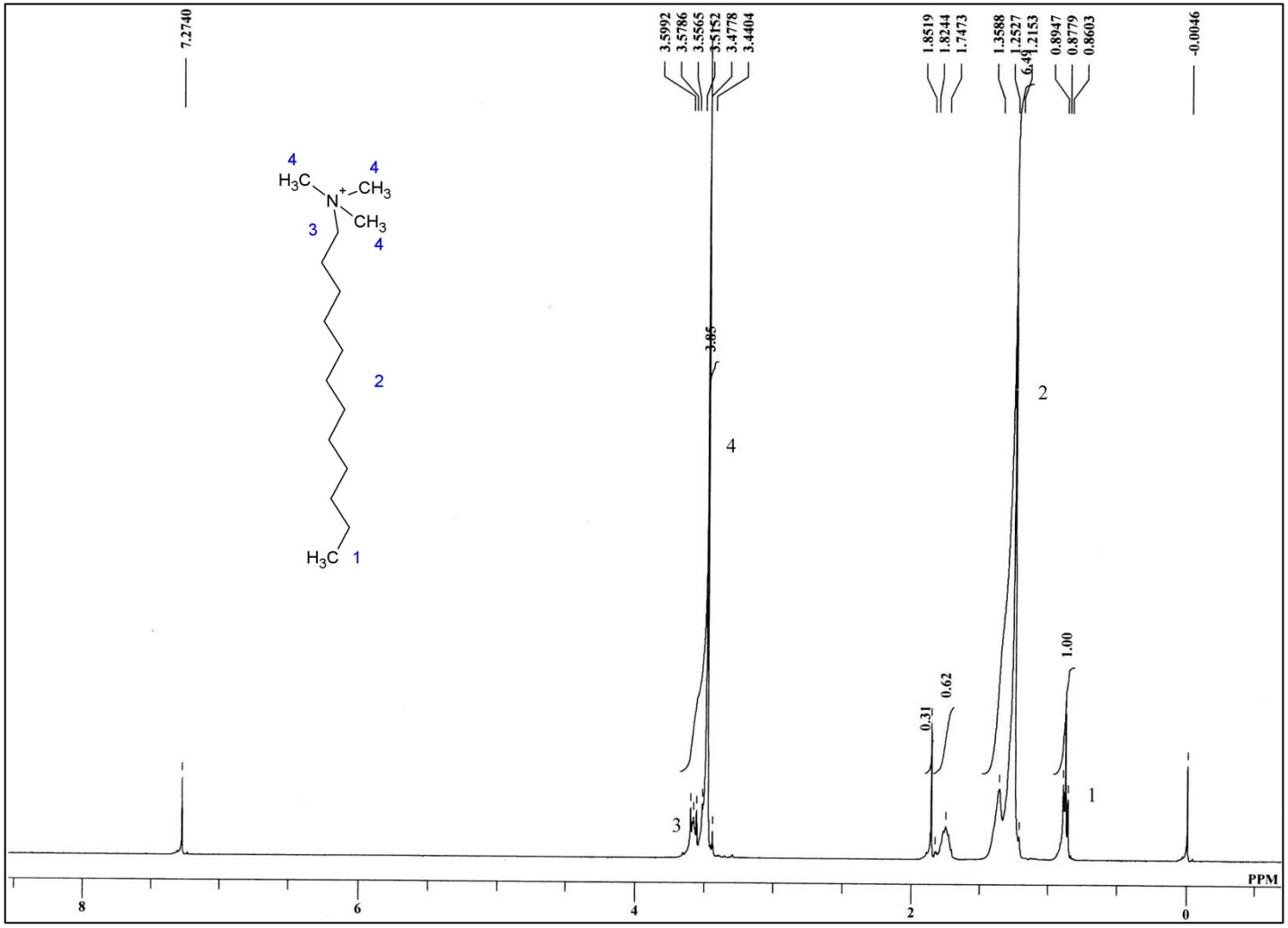
3. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2010). Synergistic Interactions in Mixed Anionic/Cationic Surfactant Mixtures: Palm-based Methyl Ester Sulfonate-Alkyl Trimethylammonium Bromide Mixtures. Oral presentation at 101st AOCS Annual Meeting and Expo, 16 – 19 May 2010, Phoenix, Arizona, United States of America.

4. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2010). Physical and Biological Properties of Palm-based Methyl Ester α-Sulfonate. Poster presented at Oils and Fats International Congress 2010 (OFIC), 20 – 22 October 2010, Kuala Lumpur Convention Centre, Kuala Lumpur.

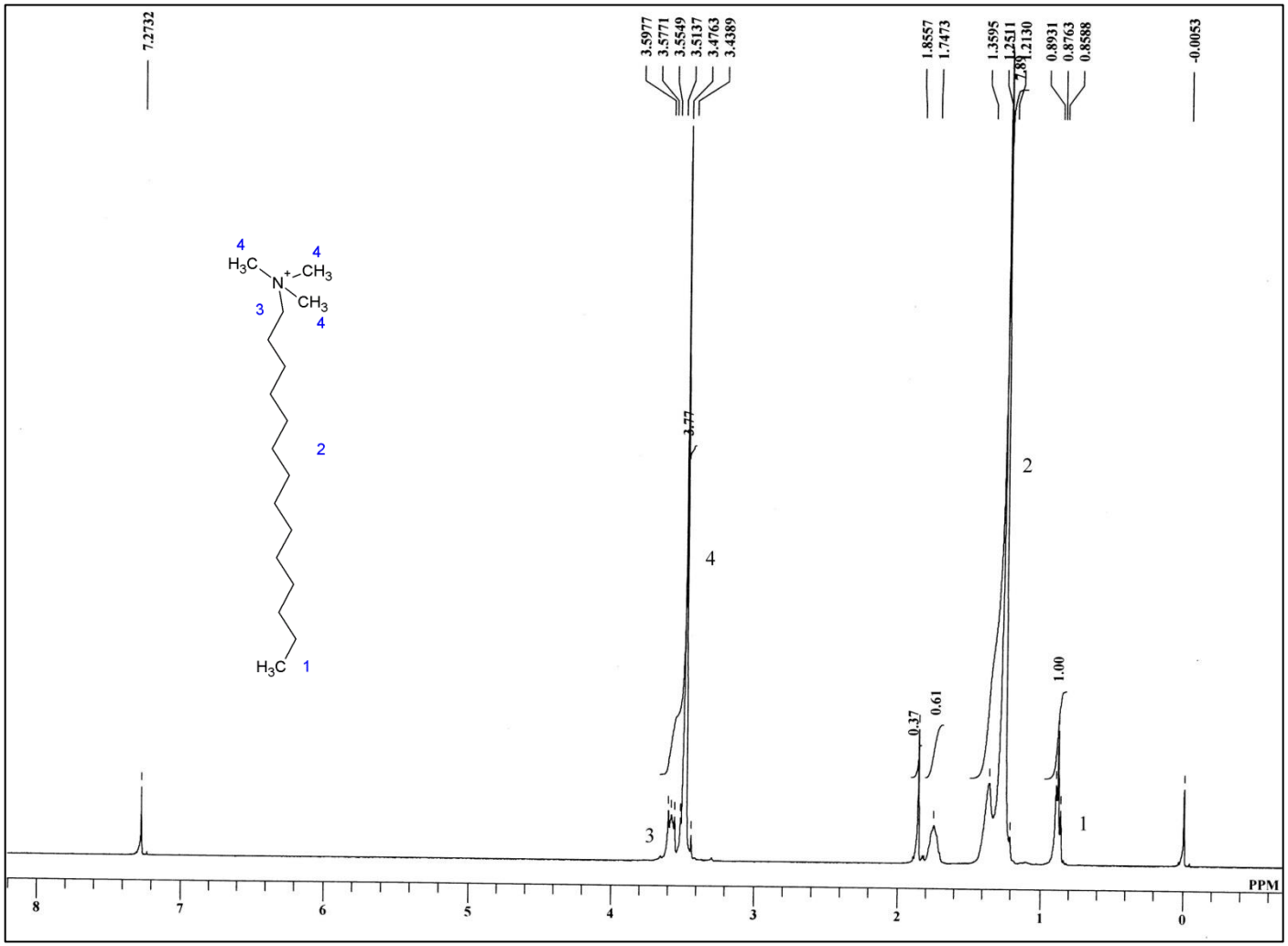
5. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2010). Physical and Biological Properties of Palm-based Methyl Ester α-Sulfonate. Poster presented at Bilateral Symposium "Emerging Trends in Chemistry" between University of Malaya and University of Hyderabad. 26-28 October 2010, Department of Chemistry, Faculty of Science, University of Malaya. Kuala Lumpur.

6. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2009). Physicochemical Properties of Anionic/Cationic Surfactant Mixtures in Dilute Solution. Poster presented at International Palm Oil Congress (PIPOC), 8 Nov to 11 Nov 2009, Kuala Lumpur Convention Centre, Kuala Lumpur.

7. Wong, S. P., Lim, W. H., Cheng, S. F. and Chuah, C. H. (2009). Surface Properties of Palm-based Catanionic Surfactant Mixtures. Poster presented at Asianalysis X Conference, 10 Aug to 13 Aug 2009, Putra World Trade Centre, Kuala Lumpur.

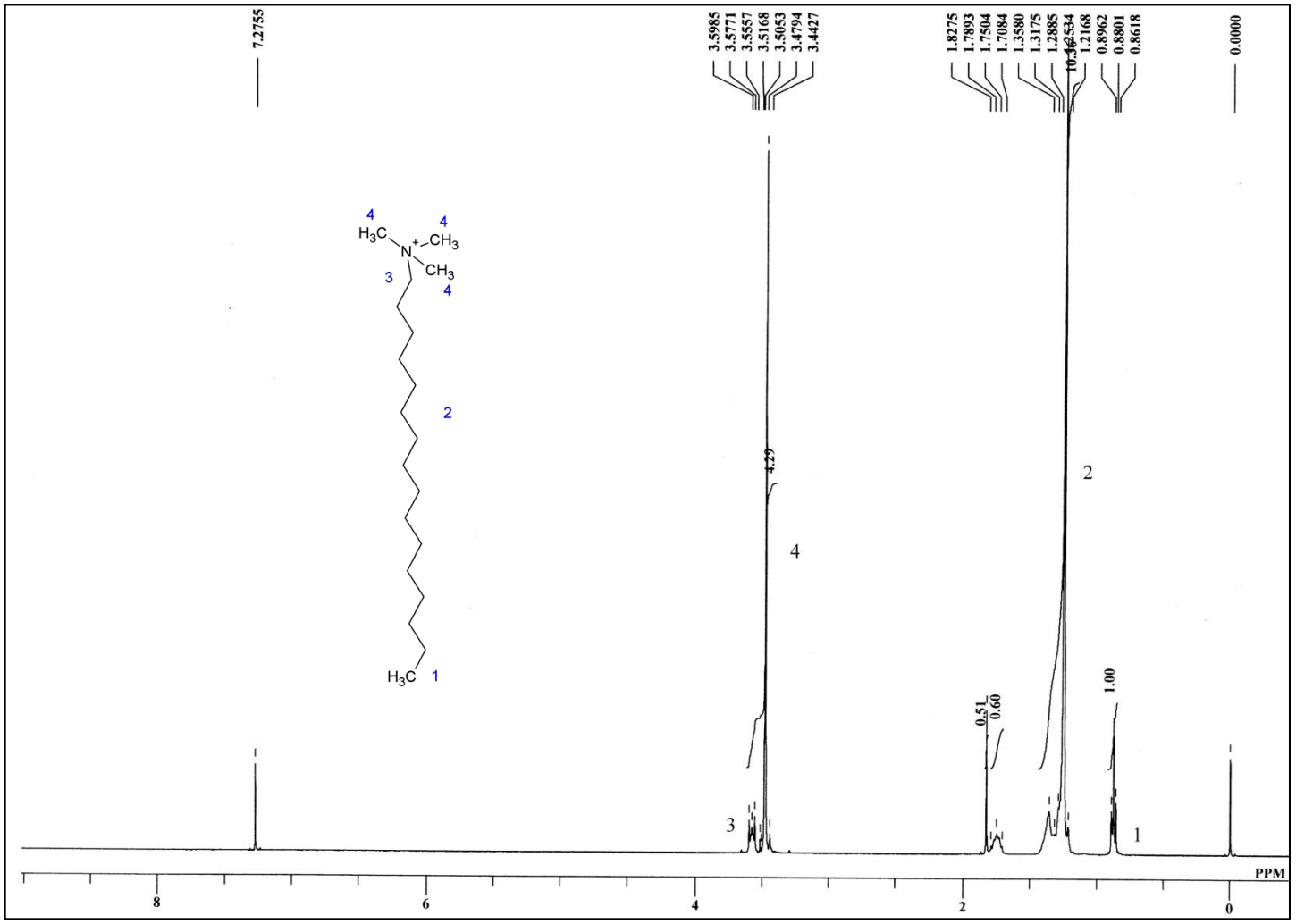


Appendix 1. ^{1}H NMR spectrum of $C_{12}TAB$ single surfactant recorded at 400 MHz.

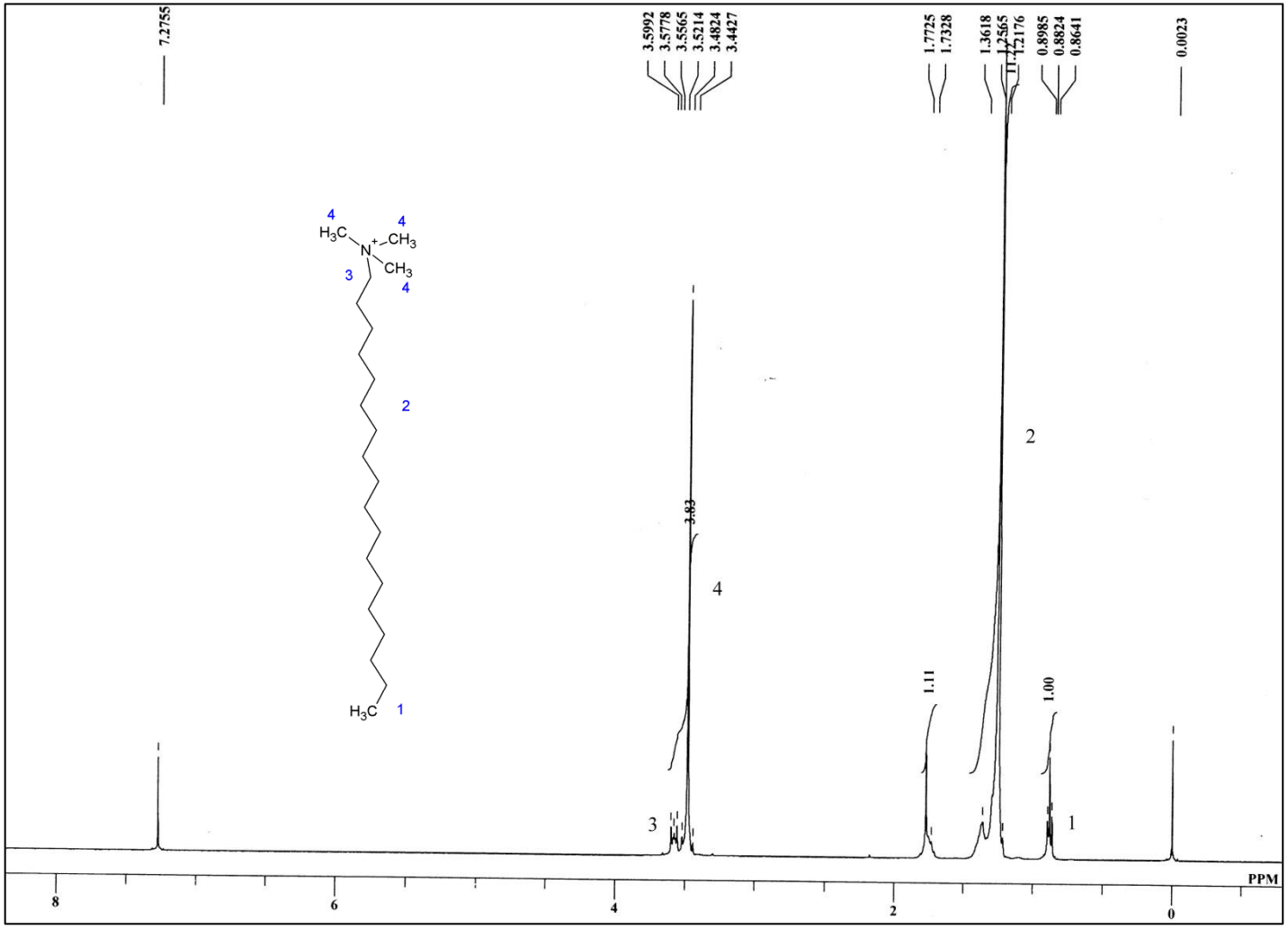


Appendix 2. ^{1}H NMR spectrum of $C_{14}TAB$ single surfactant recorded at 400 MHz.

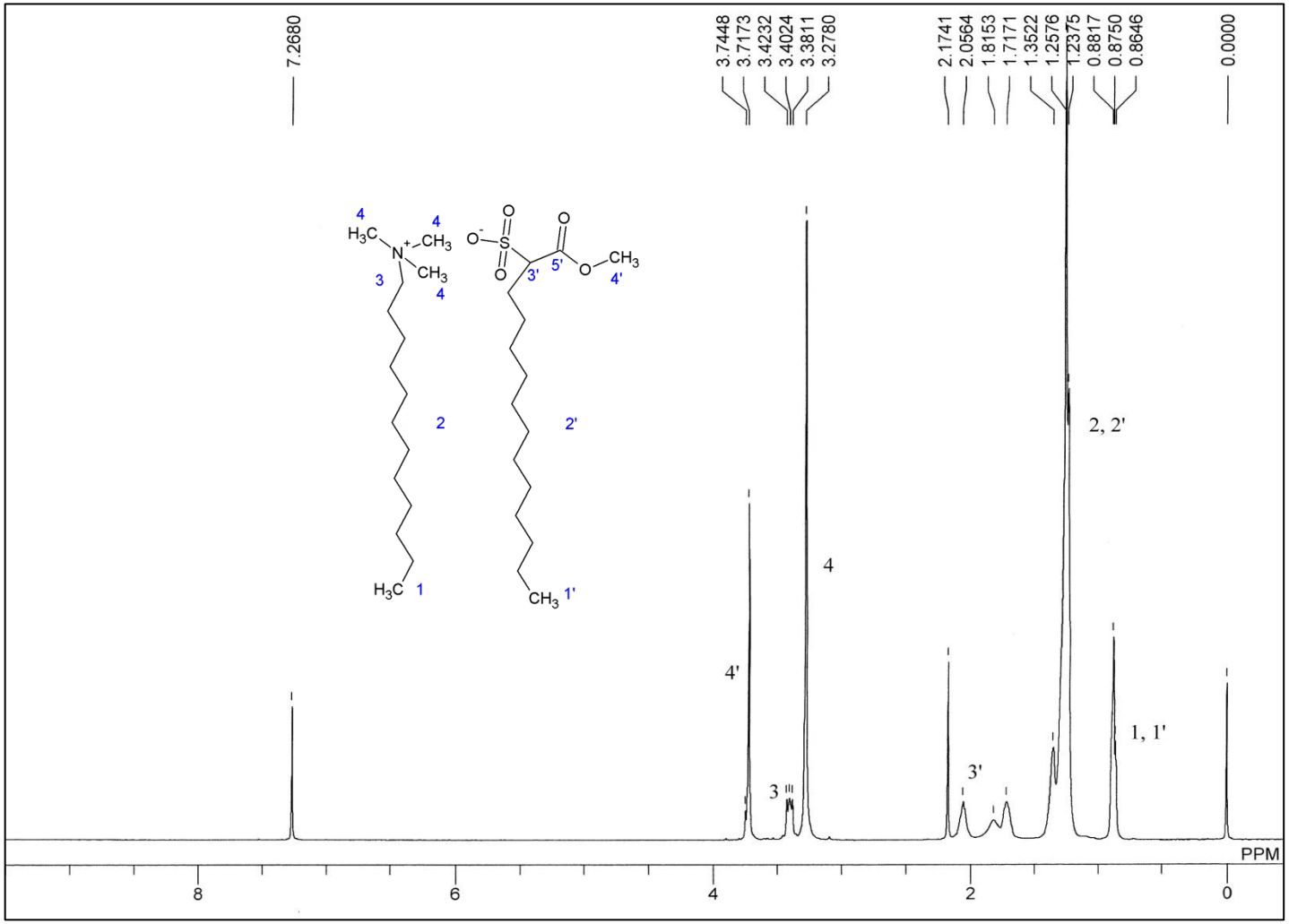
3.



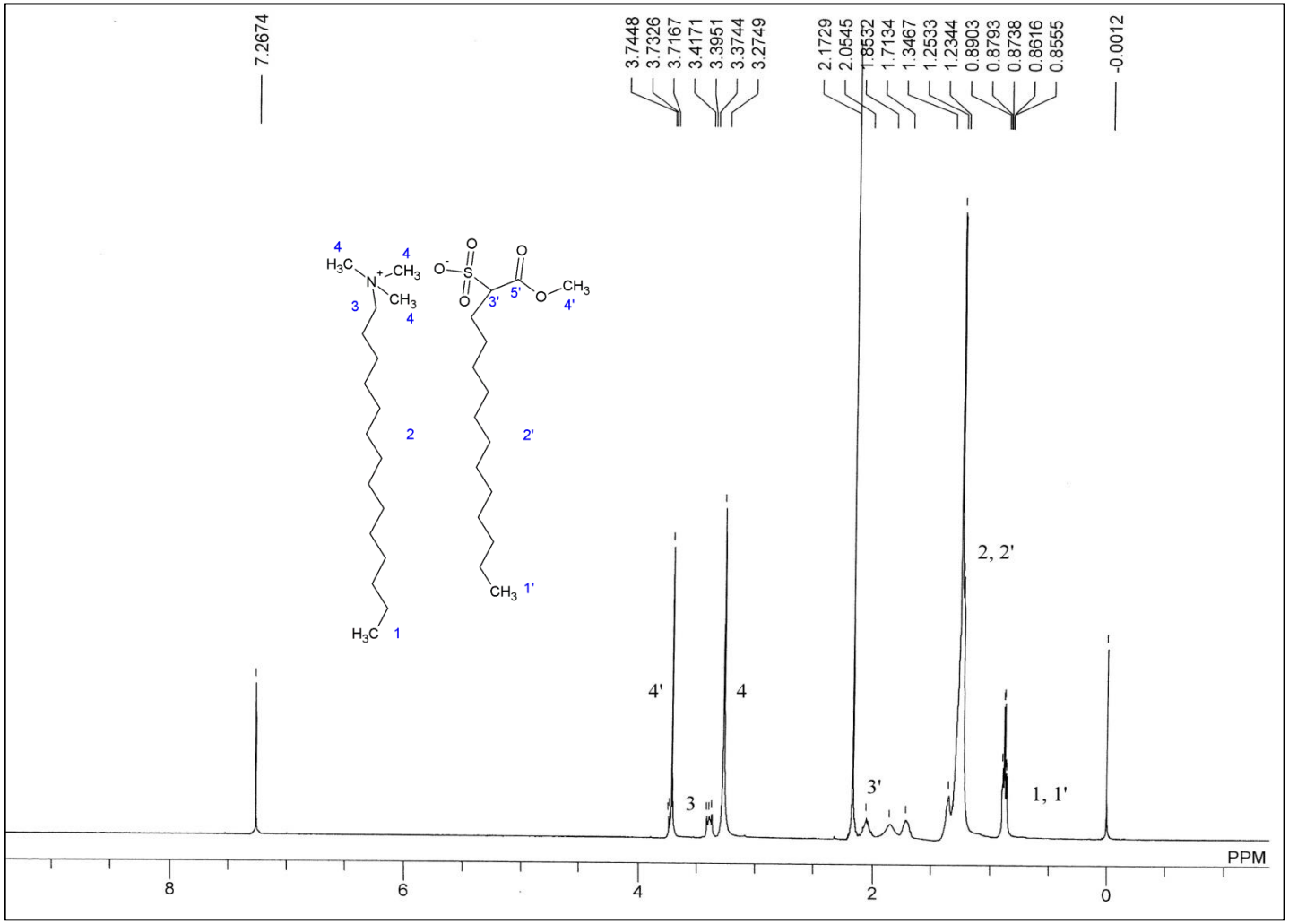
Appendix 3. ^{1}H NMR spectrum of $C_{16}TAB$ single surfactant recorded at 400 MHz.



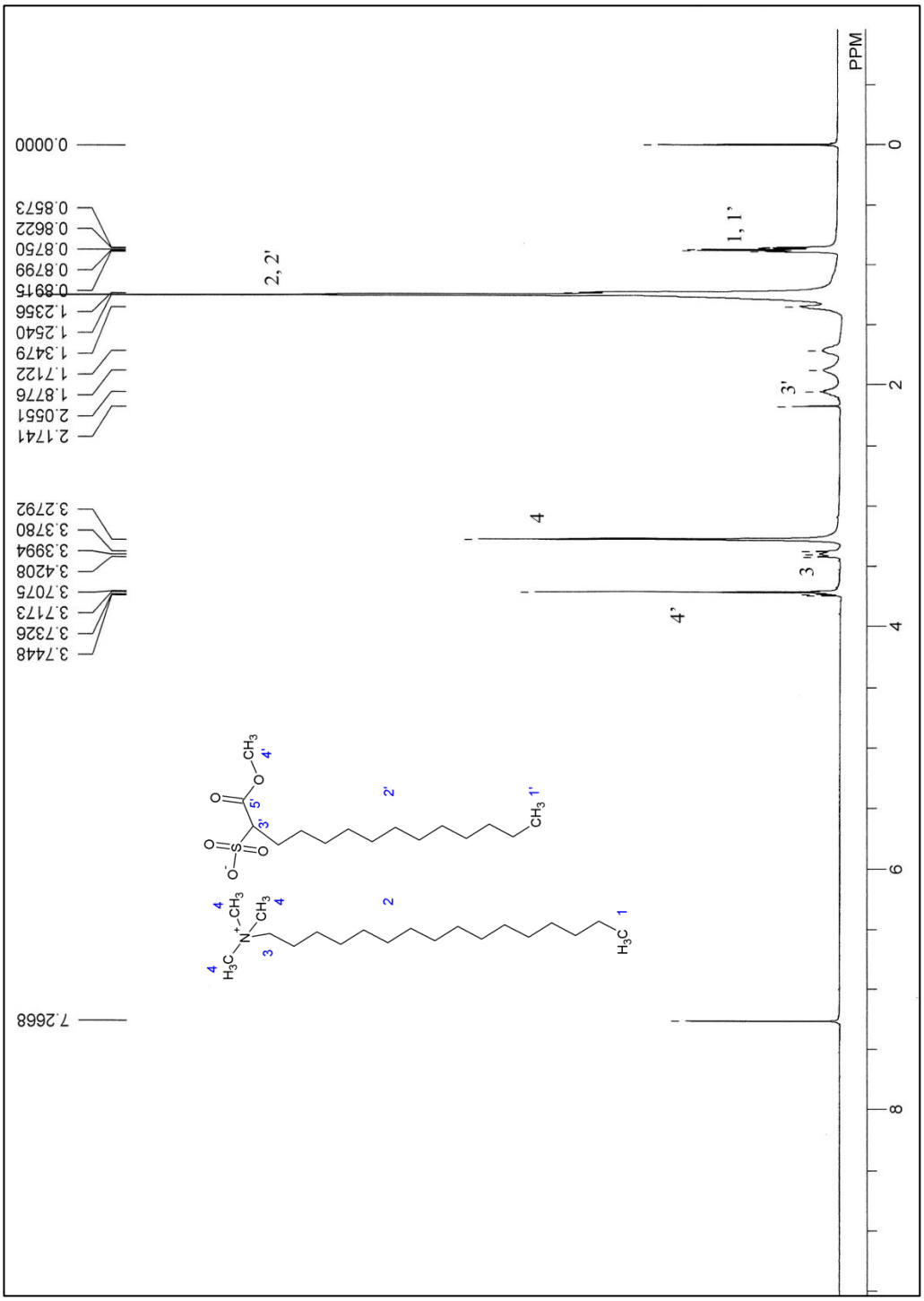
Appendix 4. ^{1}H NMR spectrum of $C_{18}TAB$ single surfactant recorded at 400 MHz.



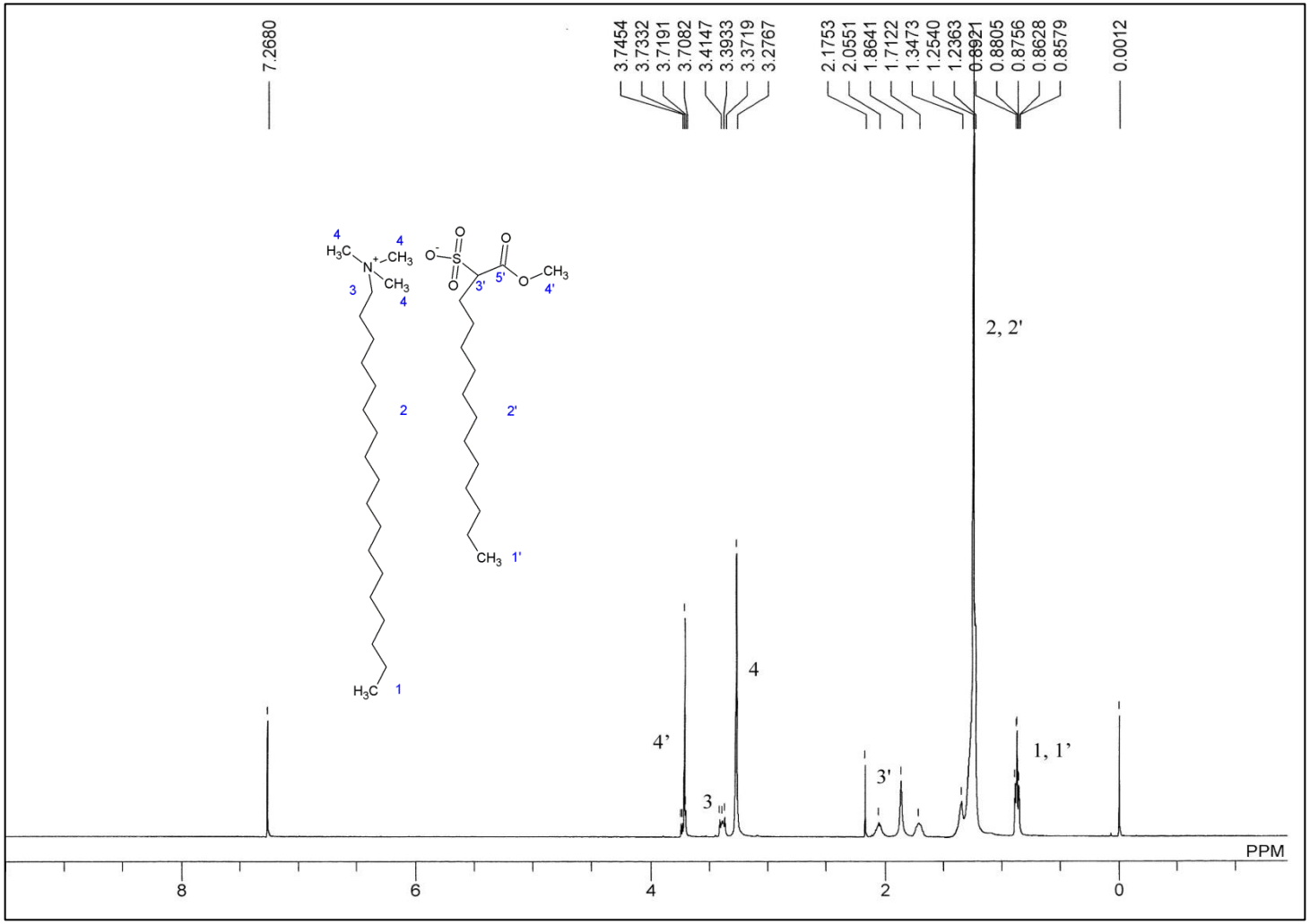
Appendix 5. ^{1}H NMR spectrum of C_{12}MES.C_{12}TAB catanionic surfactant recorded at 400 MHz.



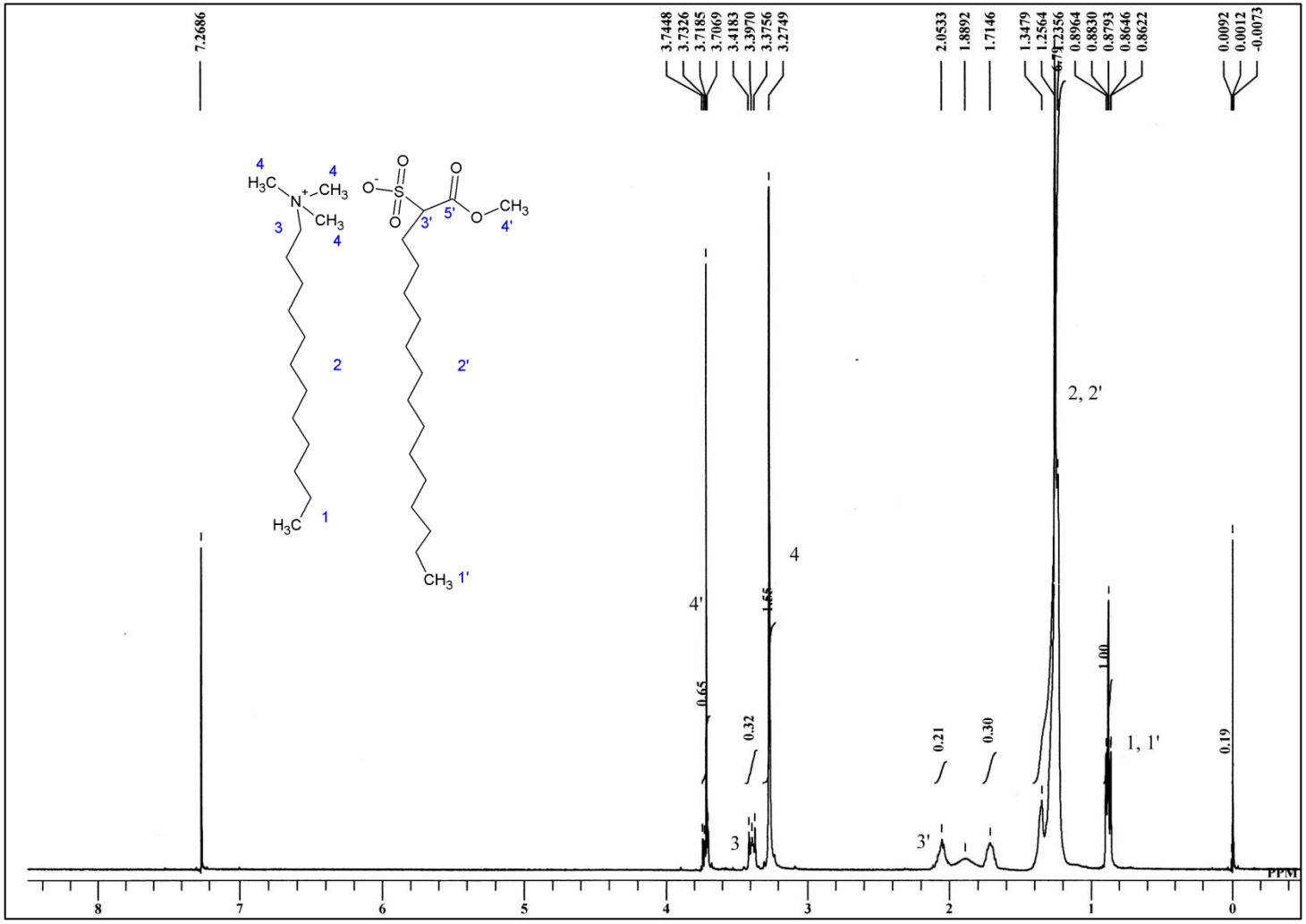
Appendix 6. ^{1}H NMR spectrum of C_{12}MES.C_{14}TAB catanionic surfactant recorded at 400 MHz.



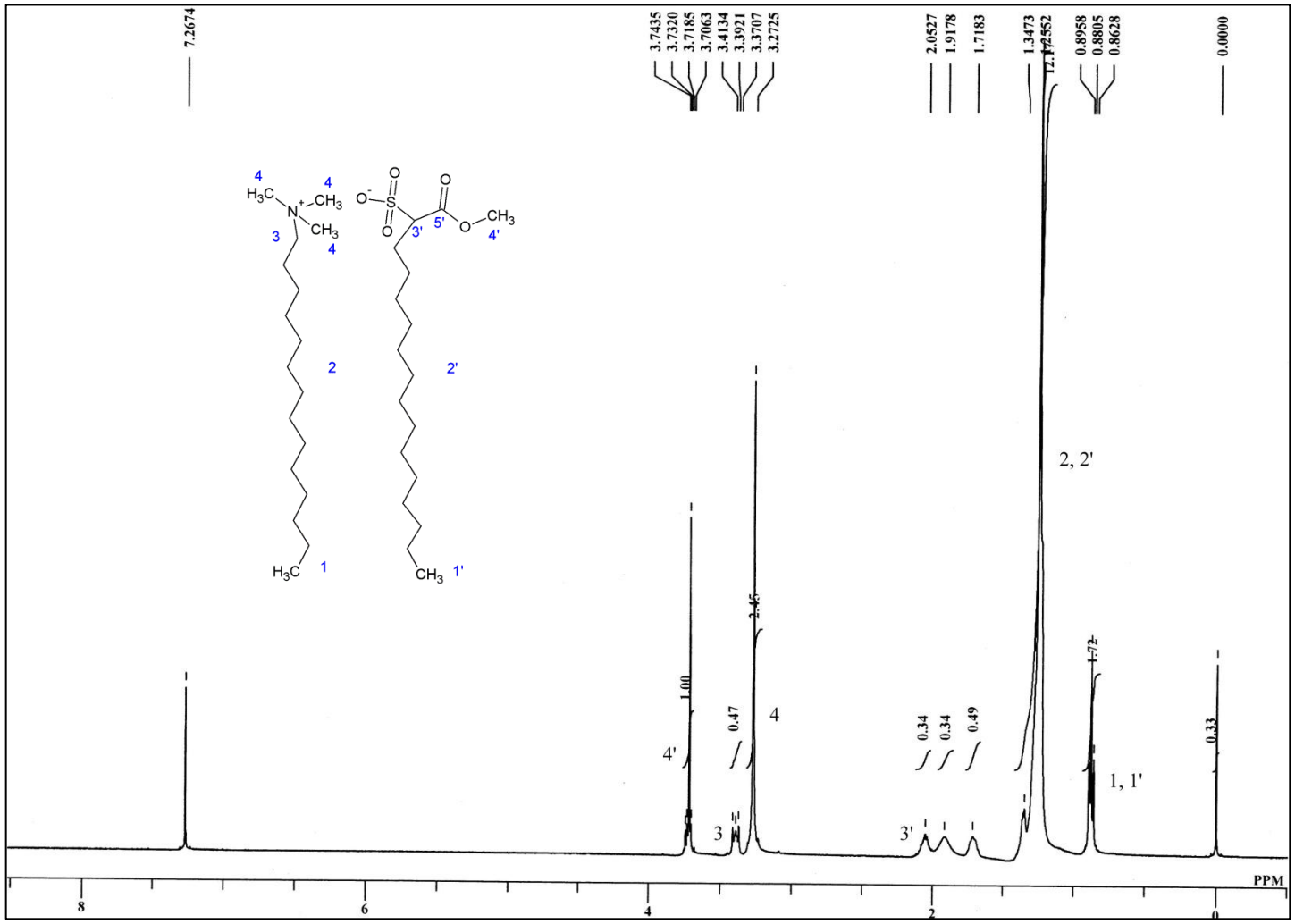
Appendix 7. ^{1}H NMR spectrum of C_{12}MES.C_{16}TAB catanionic surfactant recorded at 400 MHz.



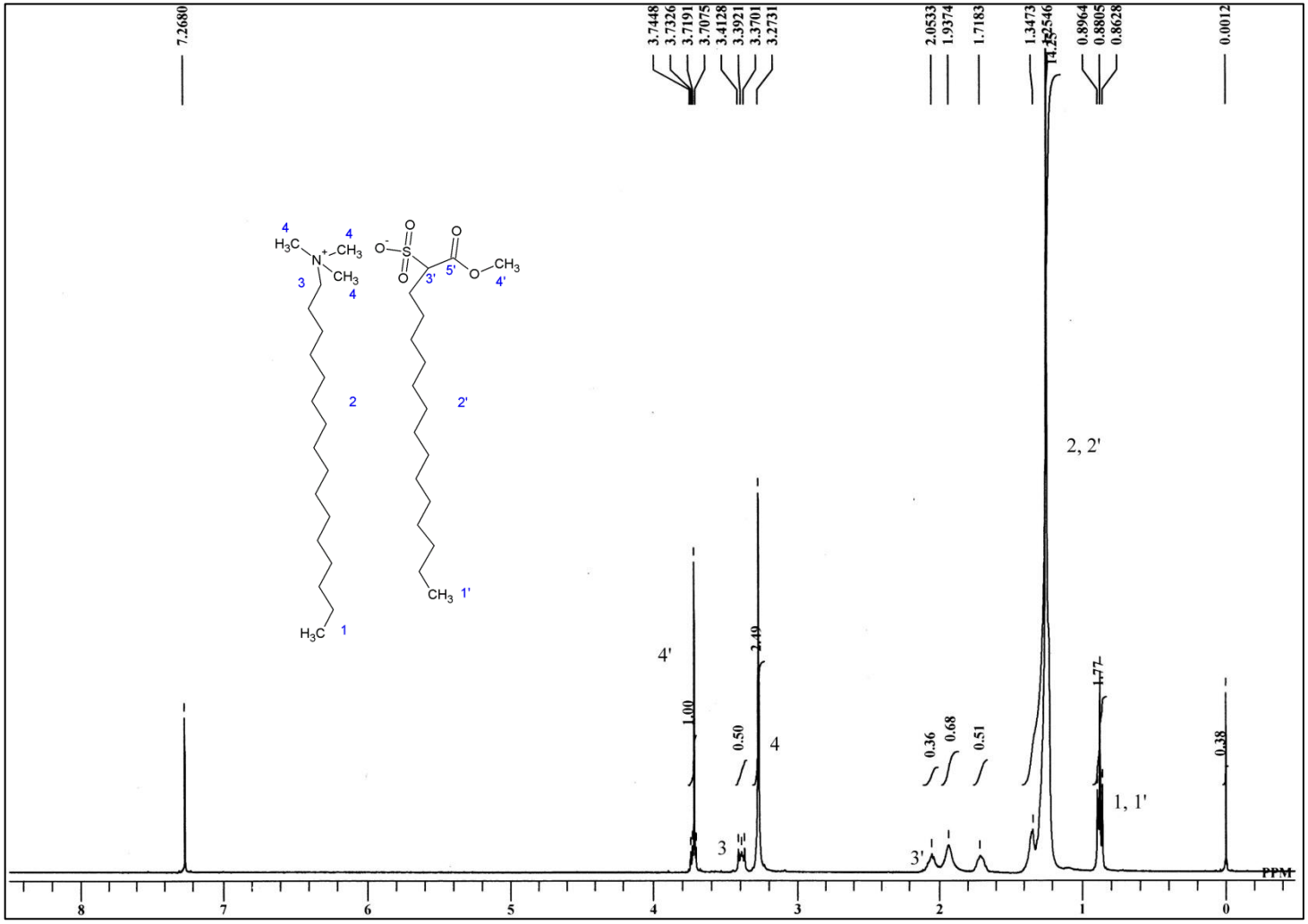
Appendix 8. ^{1}H NMR spectrum of C_{12}MES.C_{18}TAB catanionic surfactant recorded at 400 MHz.



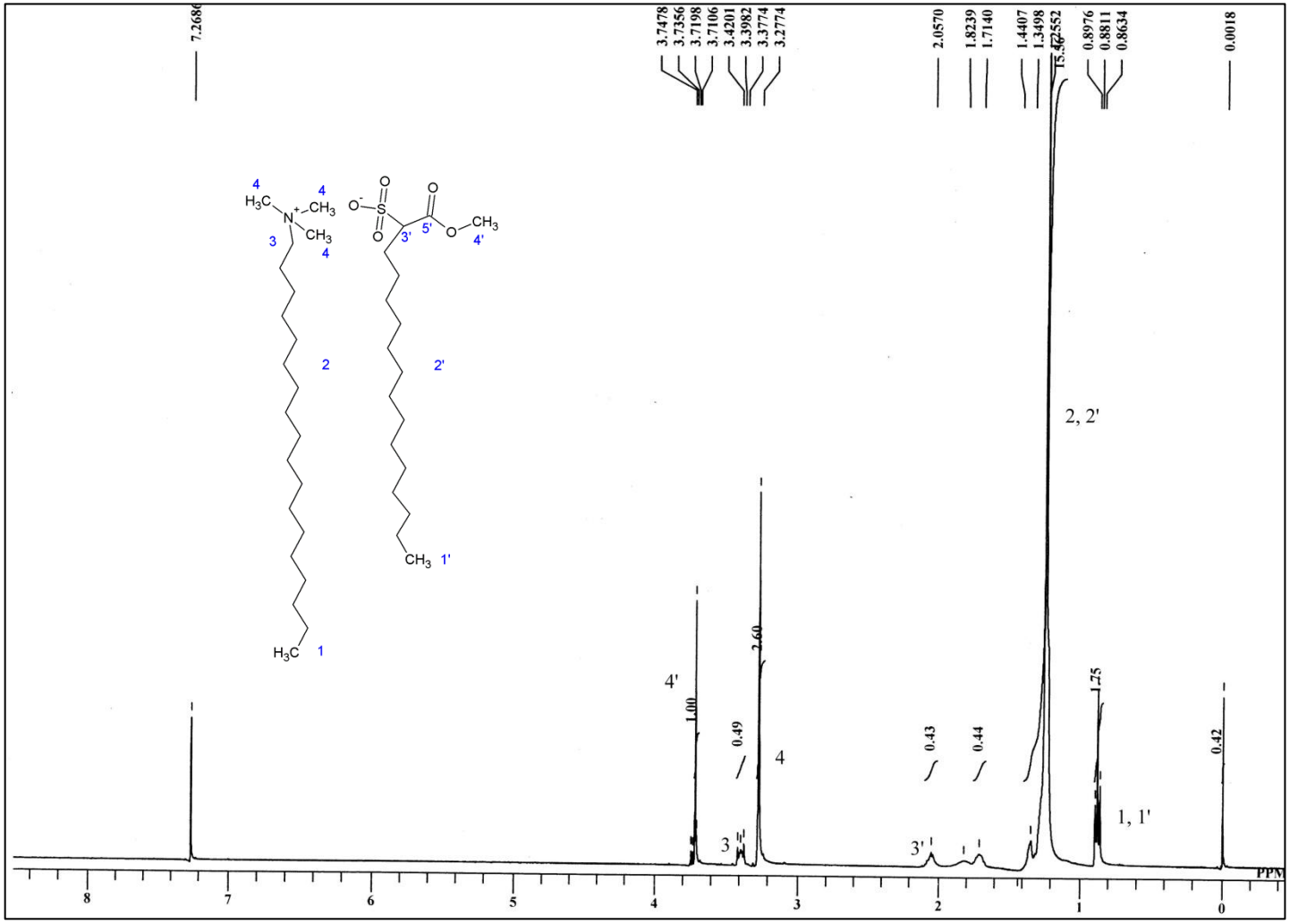
Appendix 9. ^{1}H NMR spectrum of C_{14}MES.C_{12}TAB catanionic surfactant recorded at 400 MHz.



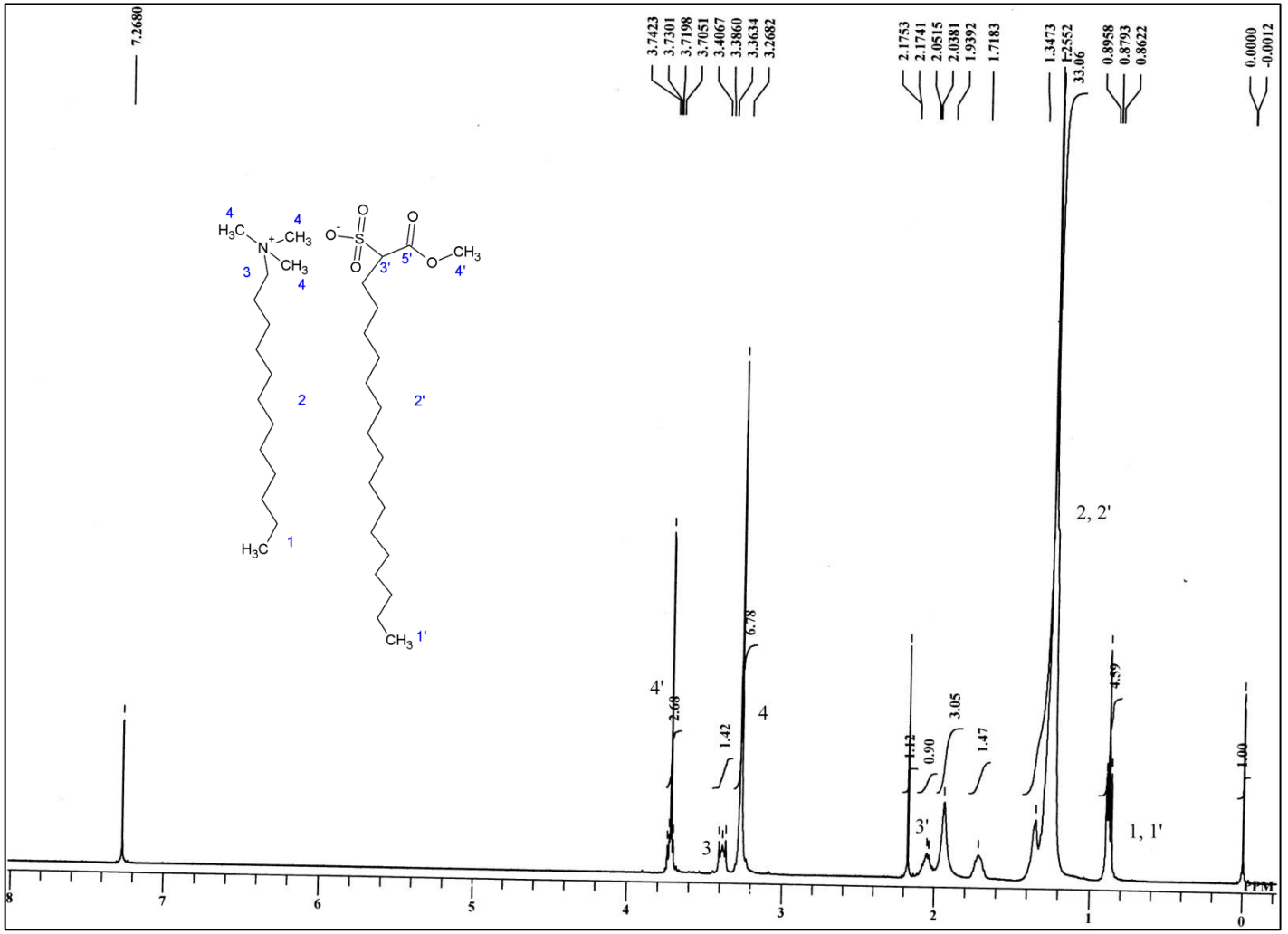
Appendix 10. ^{1}H NMR spectrum of C_{14}MES.C_{14}TAB catanionic surfactant recorded at 400 MHz.



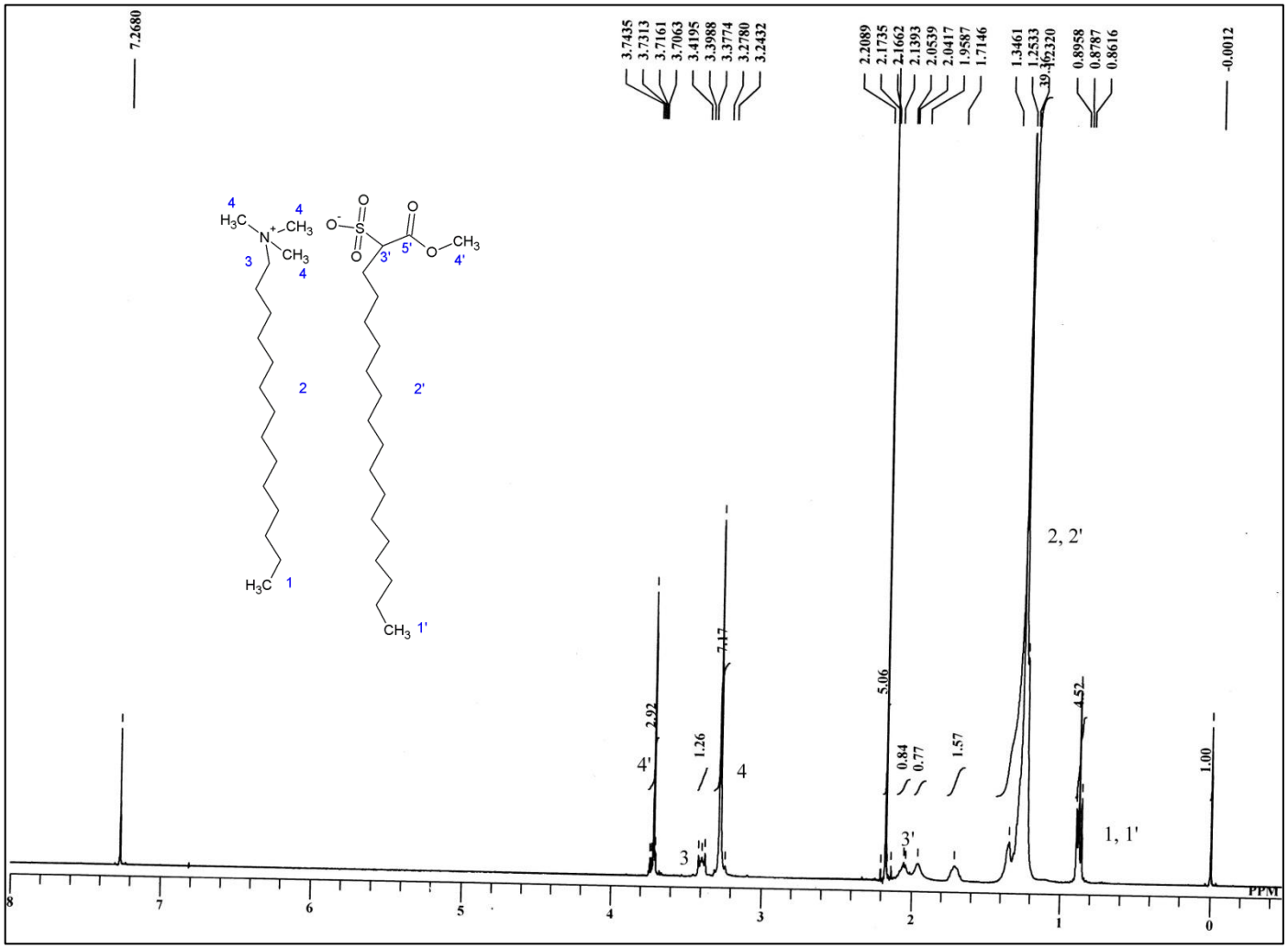
Appendix 11. ^{1}H NMR spectrum of C_{14}MES.C_{16}TAB catanionic surfactant recorded at 400 MHz.



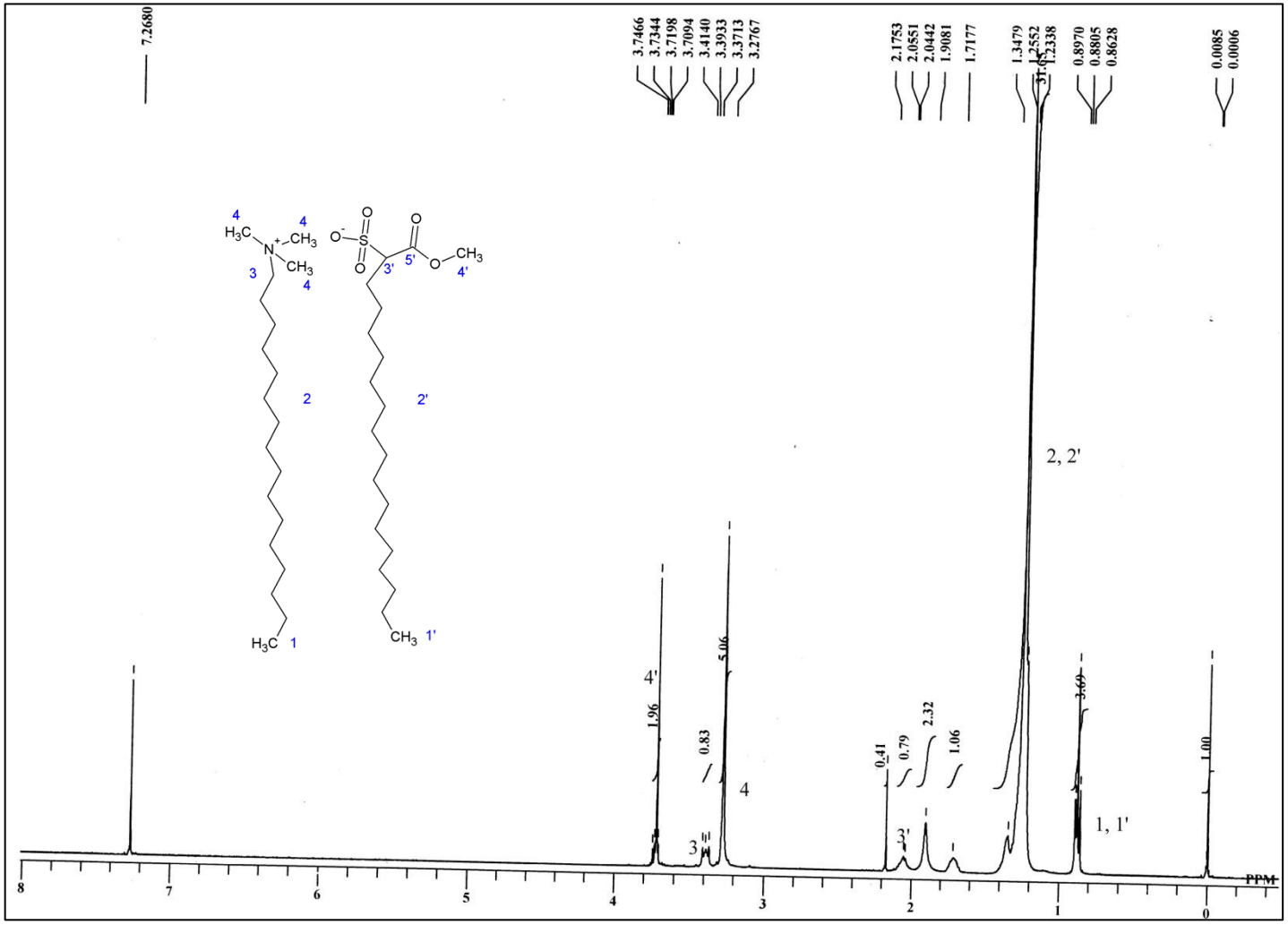
Appendix 12. ^{1}H NMR spectrum of C_{14}MES.C_{18}TAB catanionic surfactant recorded at 400 MHz.



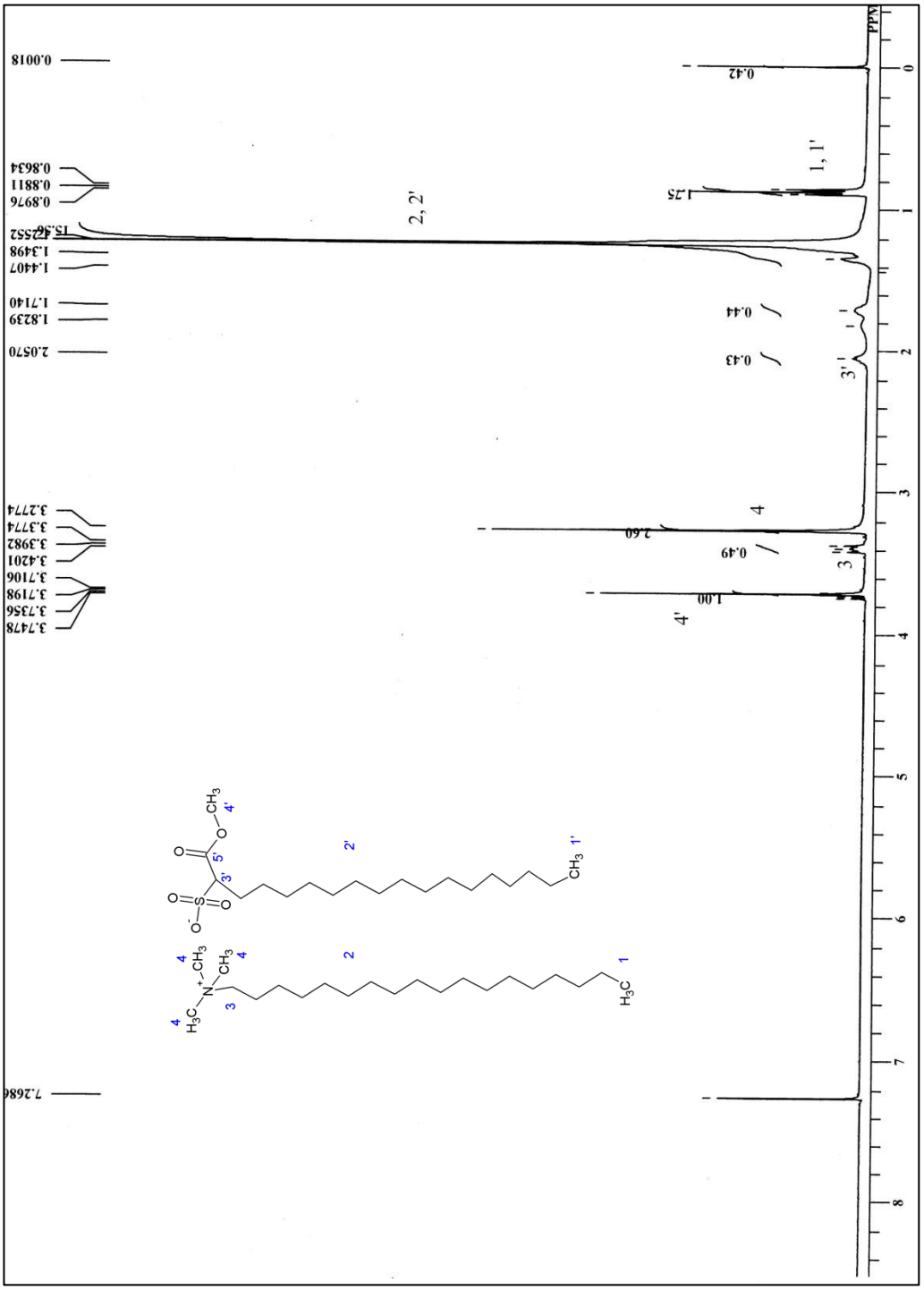
Appendix 13. ^{1}H NMR spectrum of C_{16}MES.C_{12}TAB catanionic surfactant recorded at 400 MHz.



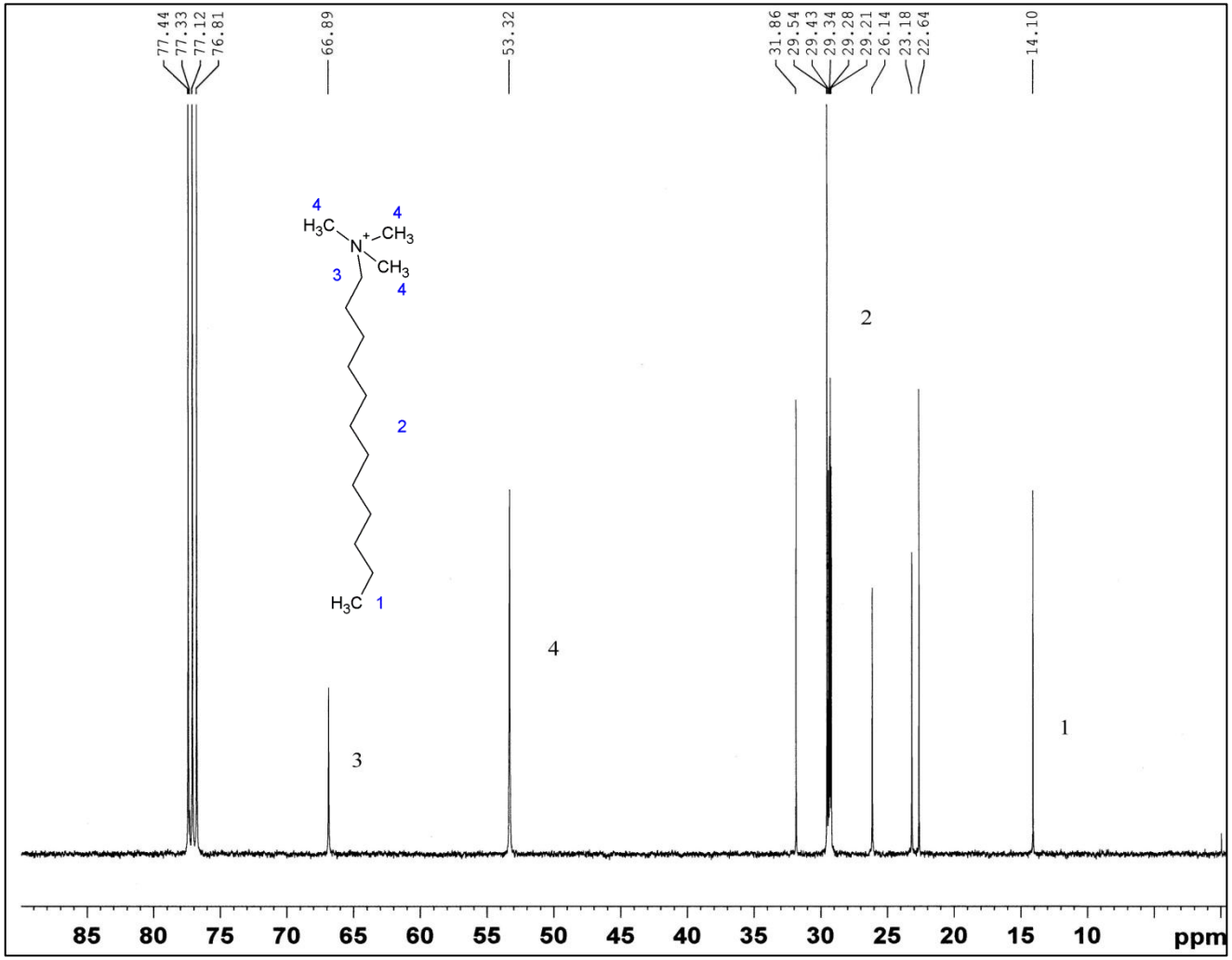
Appendix 14. ^{1}H NMR spectrum of C_{16}MES.C_{14}TAB catanionic surfactant recorded at 400 MHz.



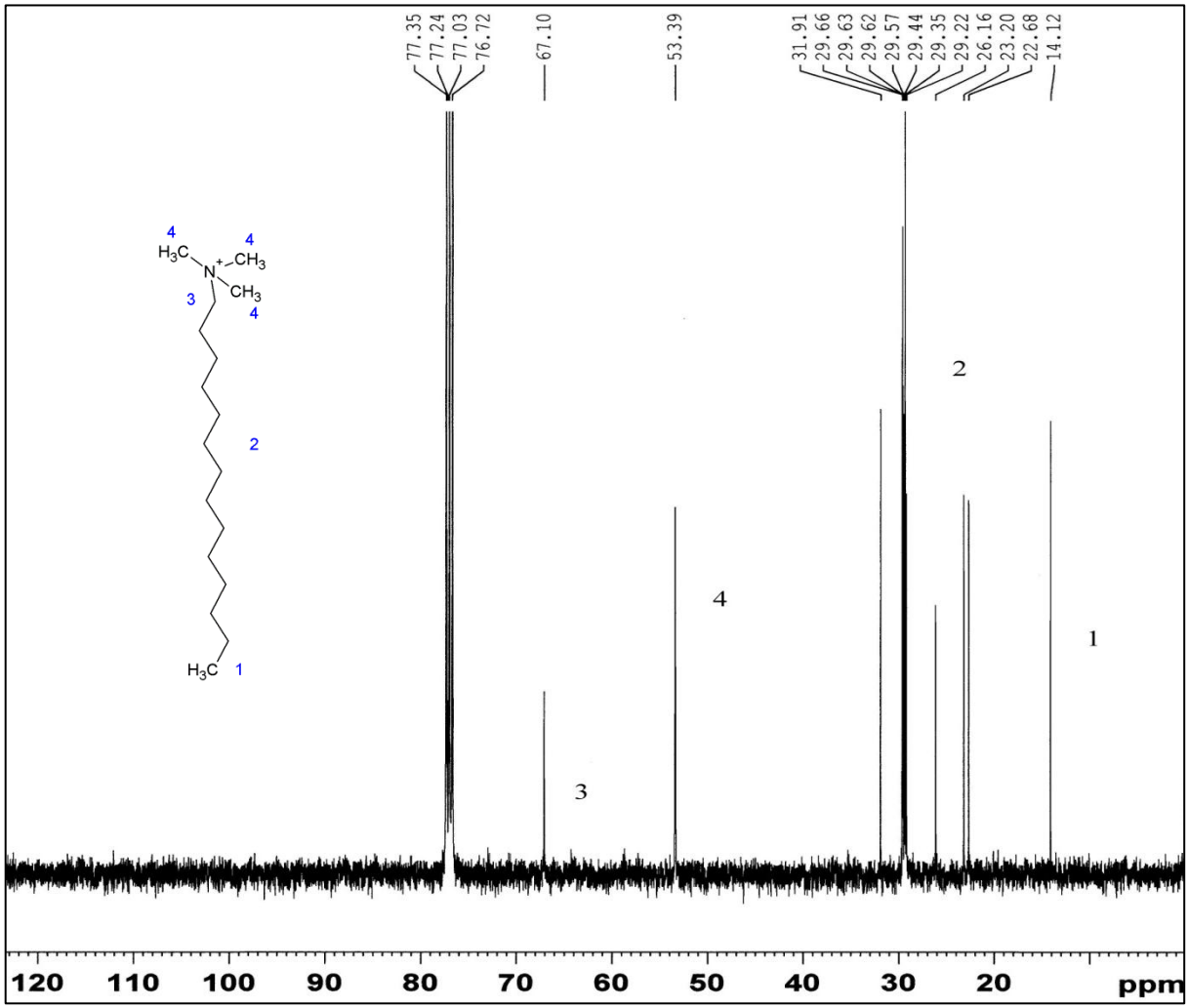
Appendix 15. ^{1}H NMR spectrum of C_{16}MES.C_{16}TAB catanionic surfactant recorded at 400 MHz.



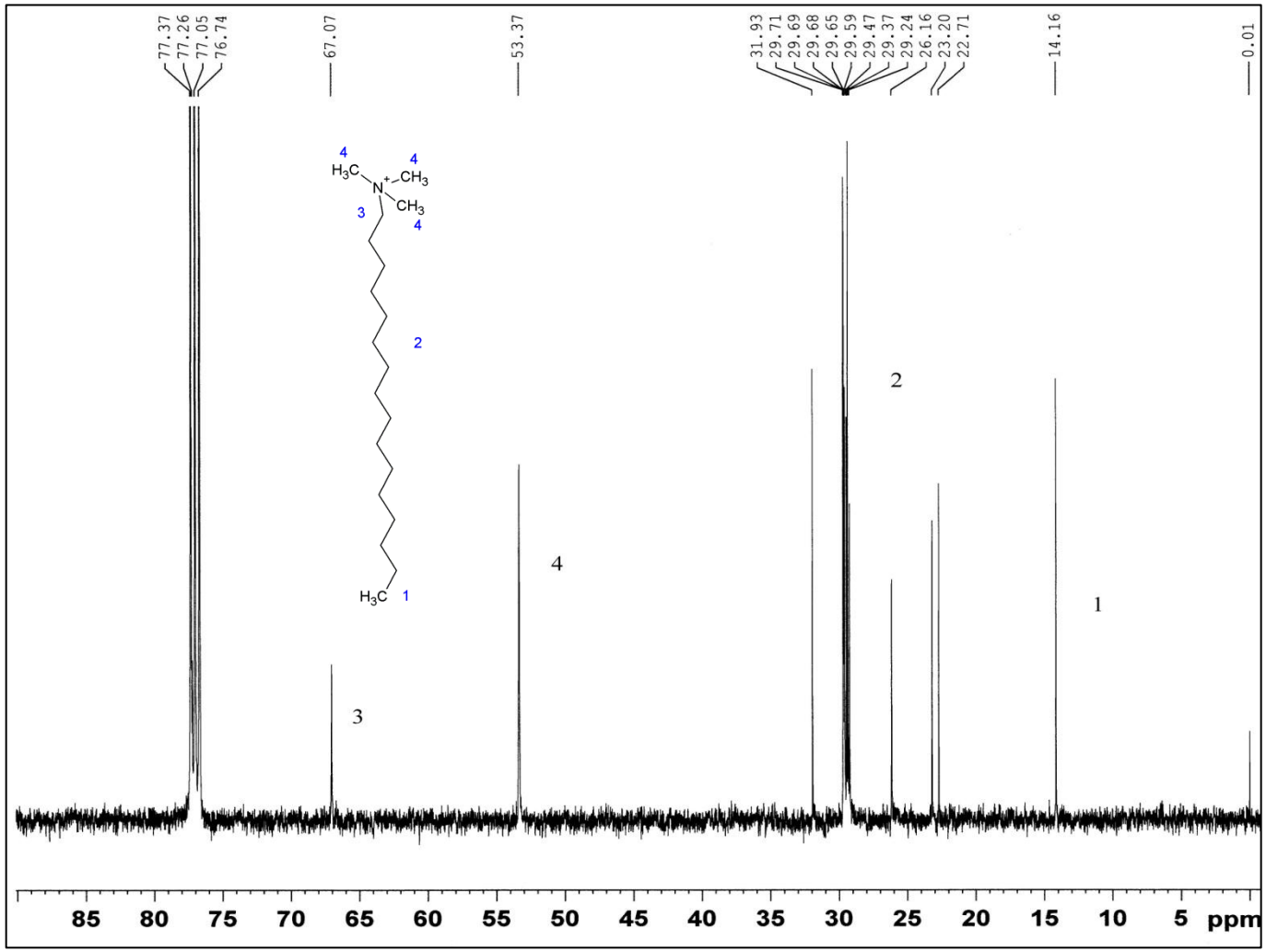
Appendix 16. ^{1}H NMR spectrum of C_{16}MES.C_{18}TAB catanionic surfactant recorded at 400 MHz.



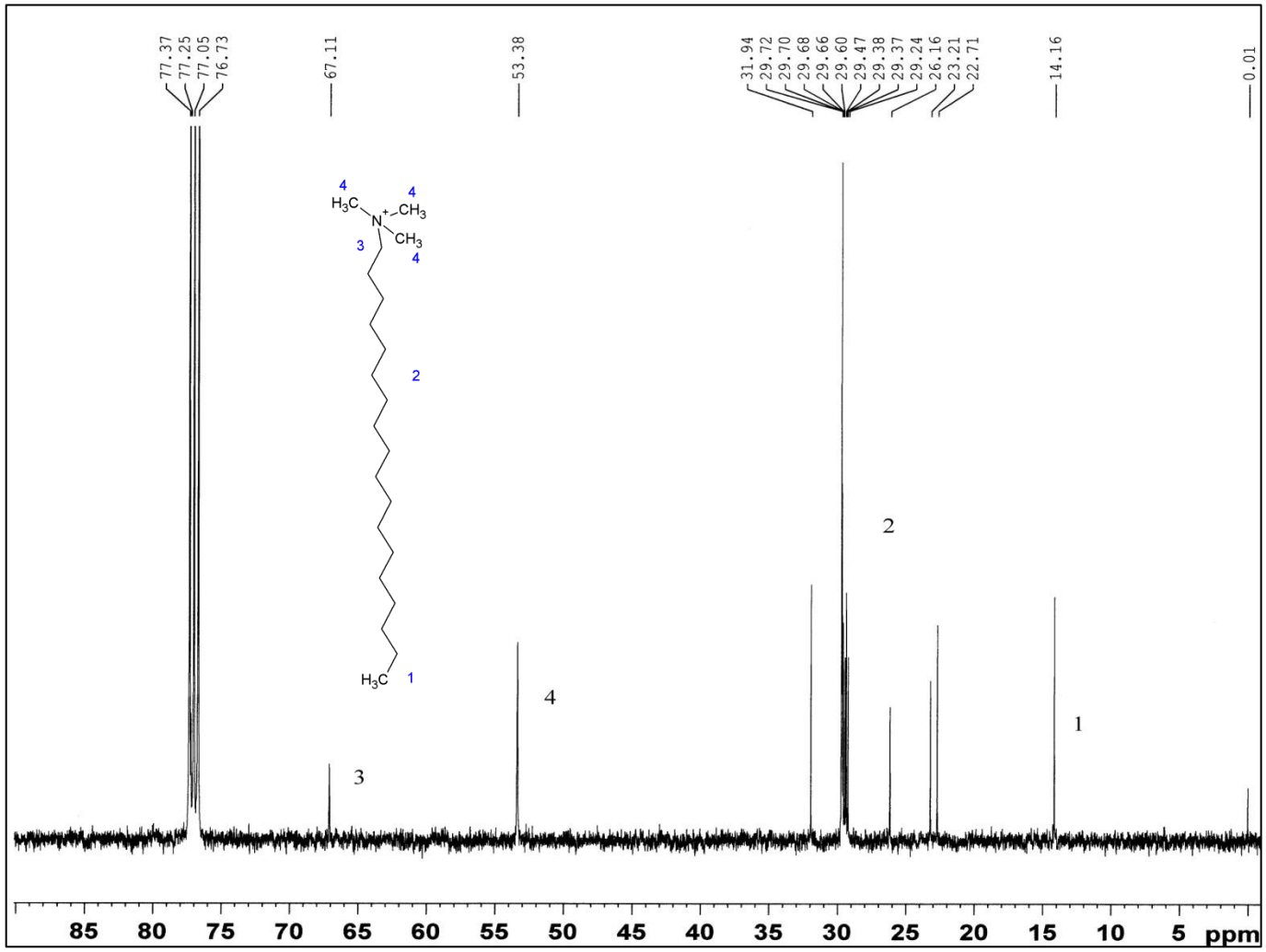
Appendix 17. ^{13}C NMR spectrum of $C_{12}TAB$ single surfactant recorded at 125 MHz.



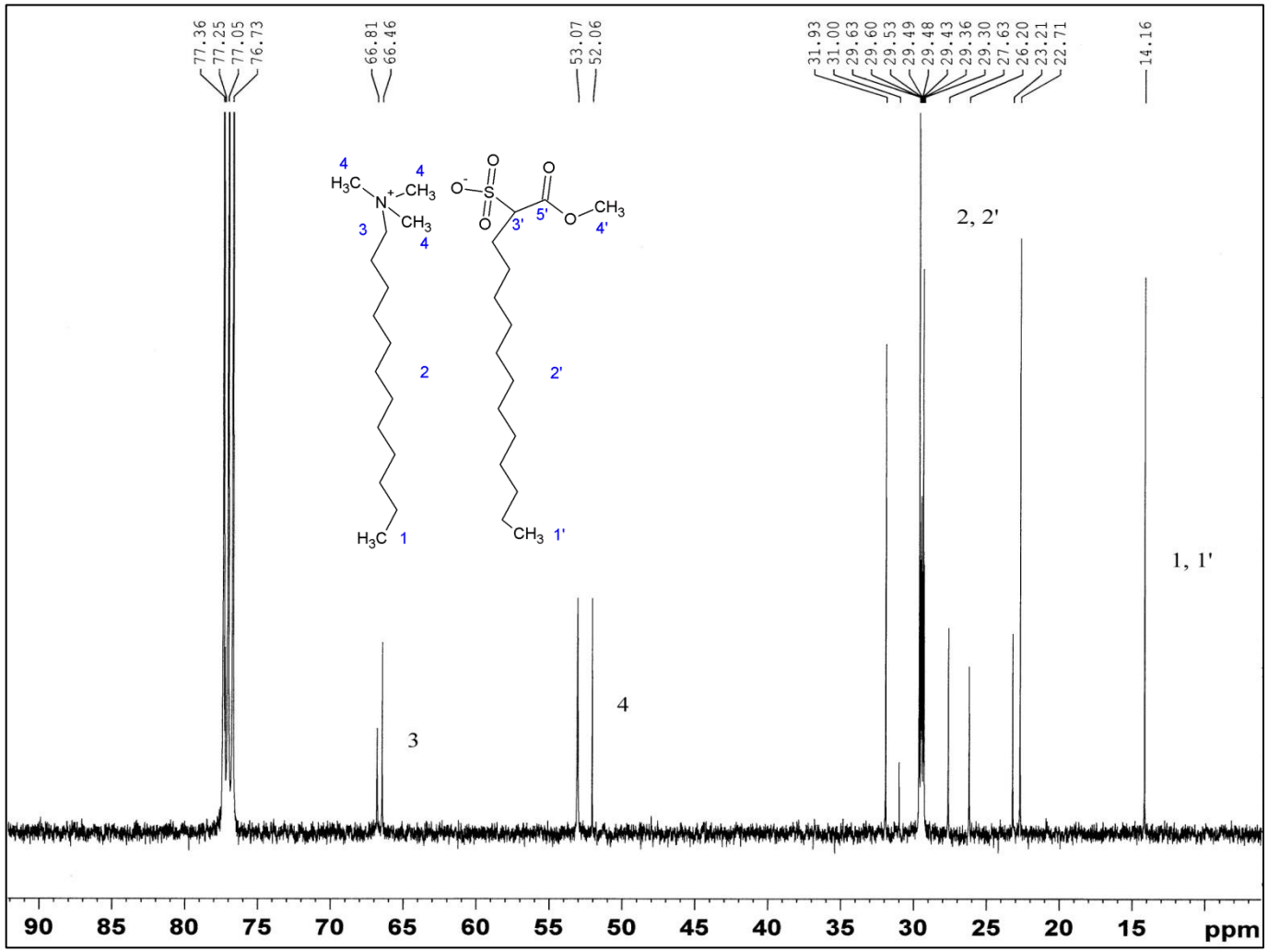
Appendix 18. ^{13}C NMR spectrum of $C_{14}TAB$ single surfactant recorded at 125 MHz.



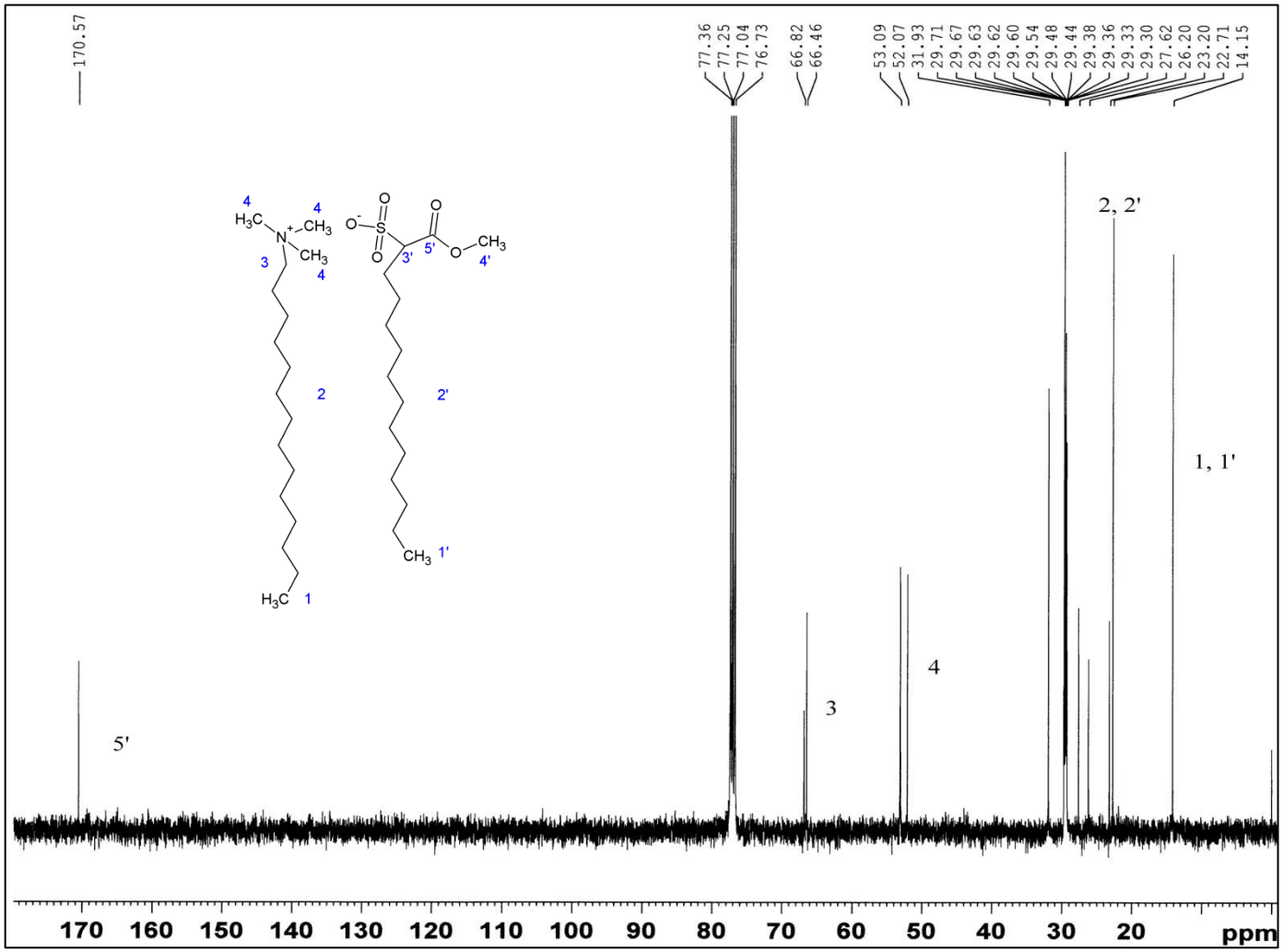
Appendix 19. ^{13}C NMR spectrum of $C_{16}TAB$ single surfactant recorded at 125 MHz.



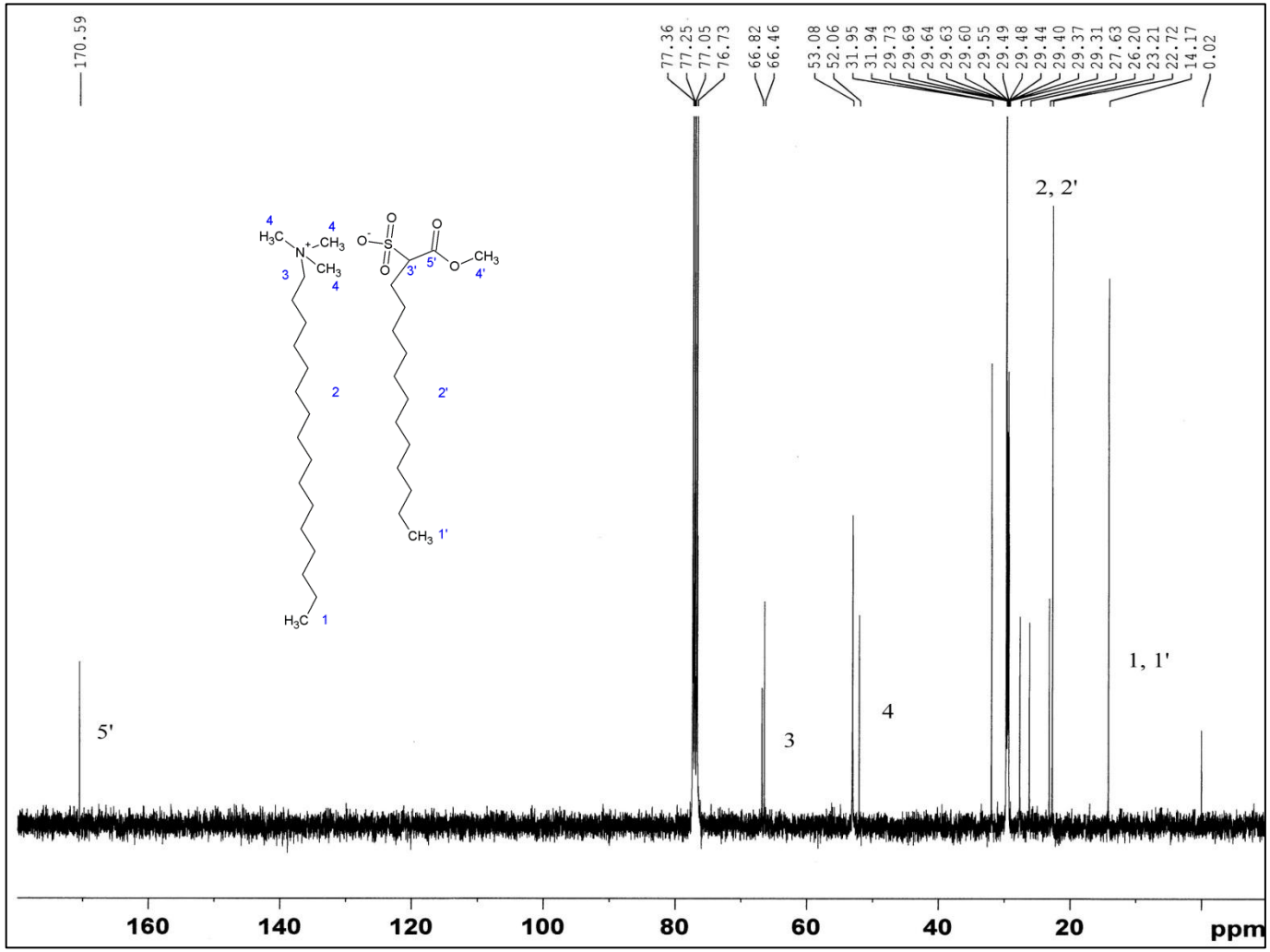
Appendix 20. ^{13}C NMR spectrum of $C_{18}TAB$ single surfactant recorded at 125 MHz.



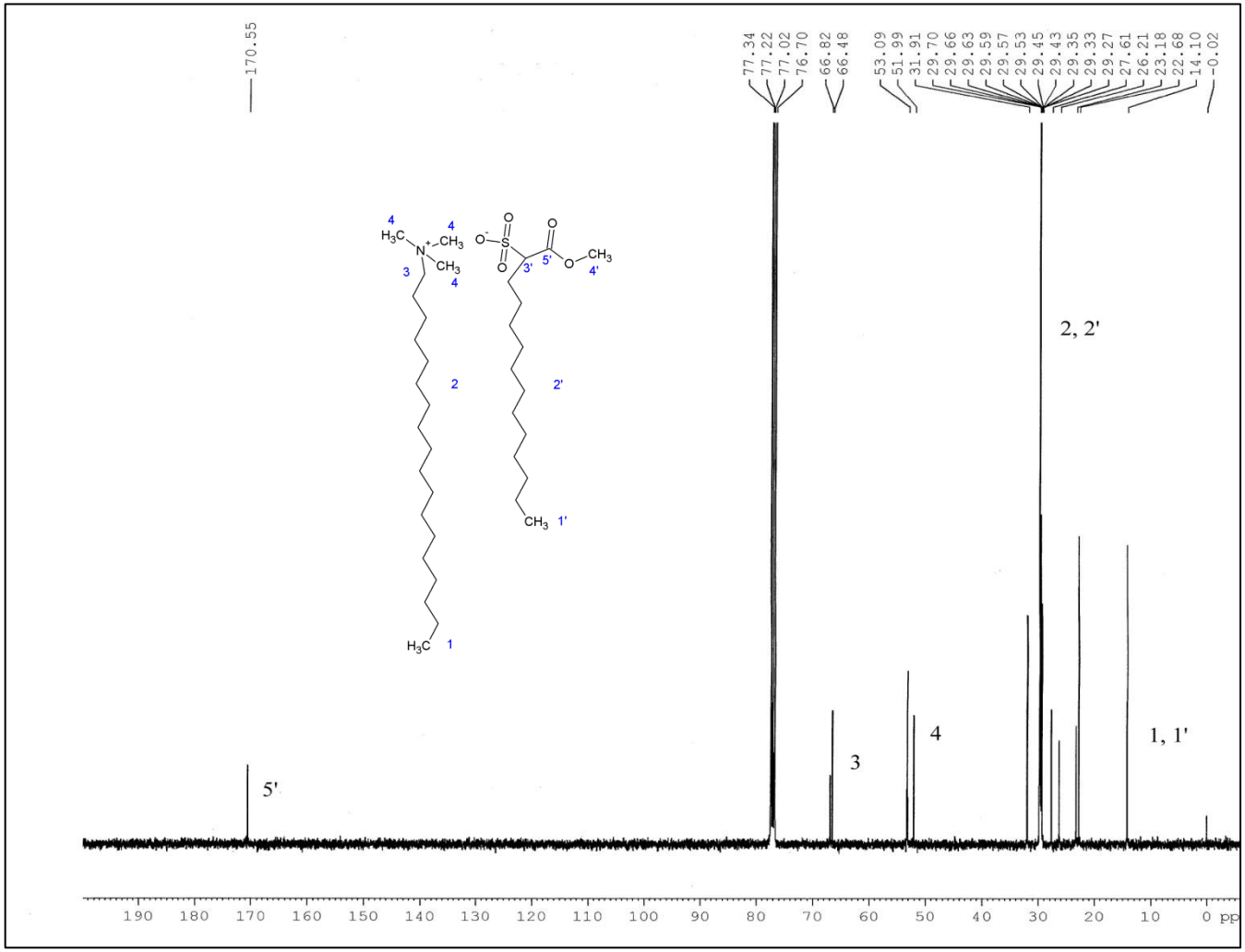
Appendix 21. ^{13}C NMR spectrum of $C_{12}MES.C_{12}TAB$ catanionic surfactant recorded at 125 MHz.



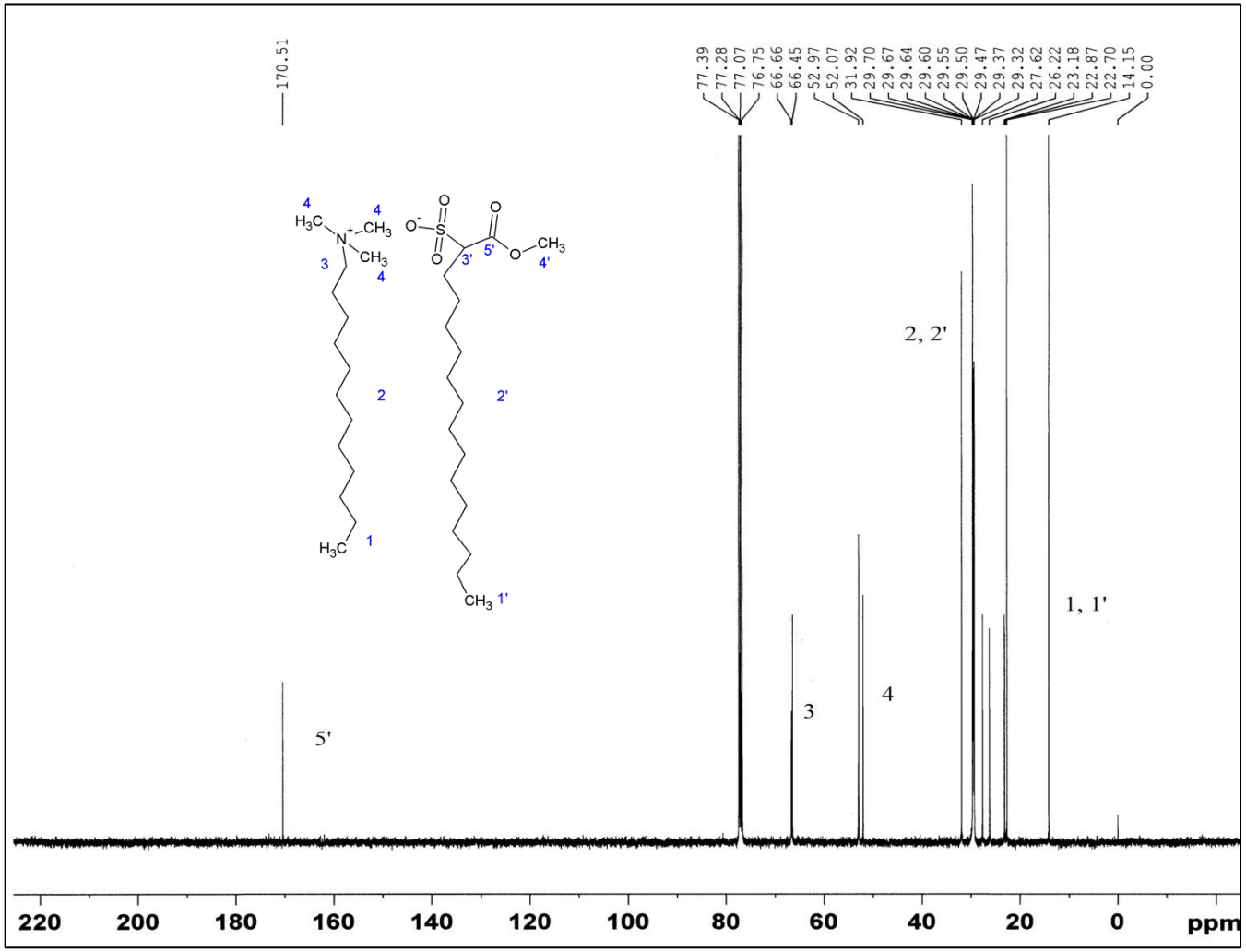
Appendix 22. ^{13}C NMR spectrum of $C_{12}MES.C_{14}TAB$ catanionic surfactant recorded at 125 MHz.



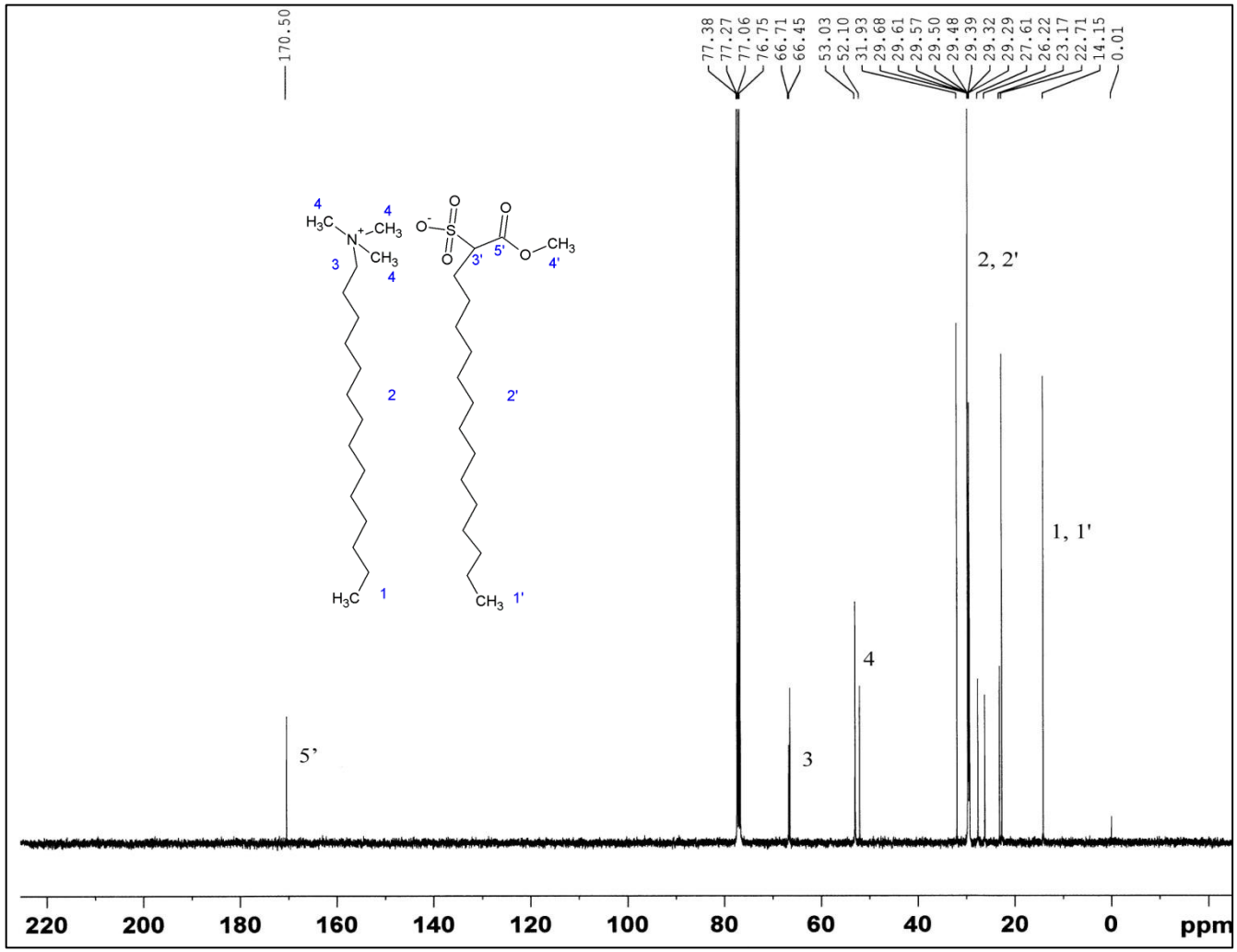
Appendix 23. ^{13}C NMR spectrum of $C_{12}MES.C_{16}TAB$ catanionic surfactant recorded at 125 MHz.



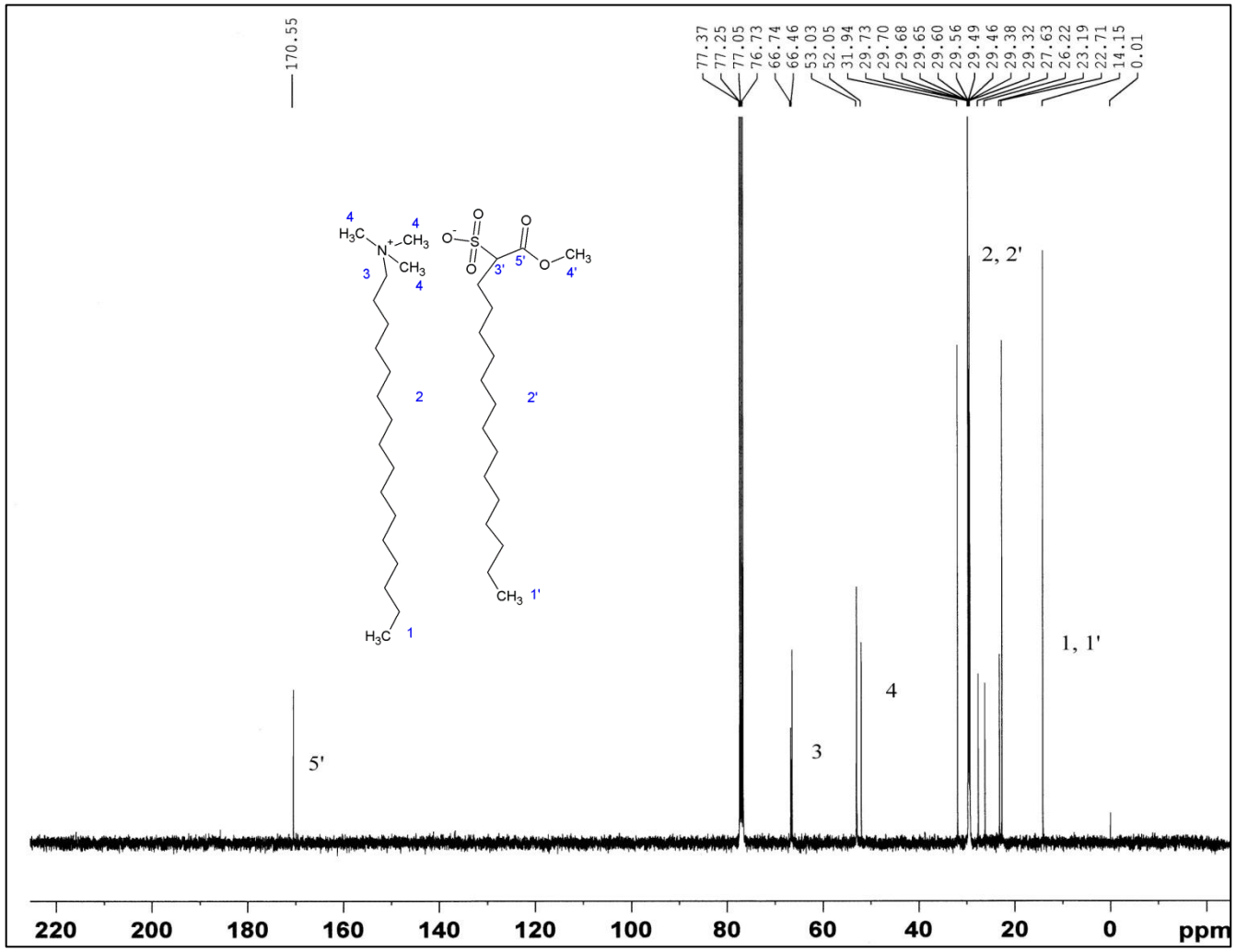
Appendix 24. ^{13}C NMR spectrum of C_{12}MES.C_{18}TAB catanionic surfactant recorded at 125 MHz.



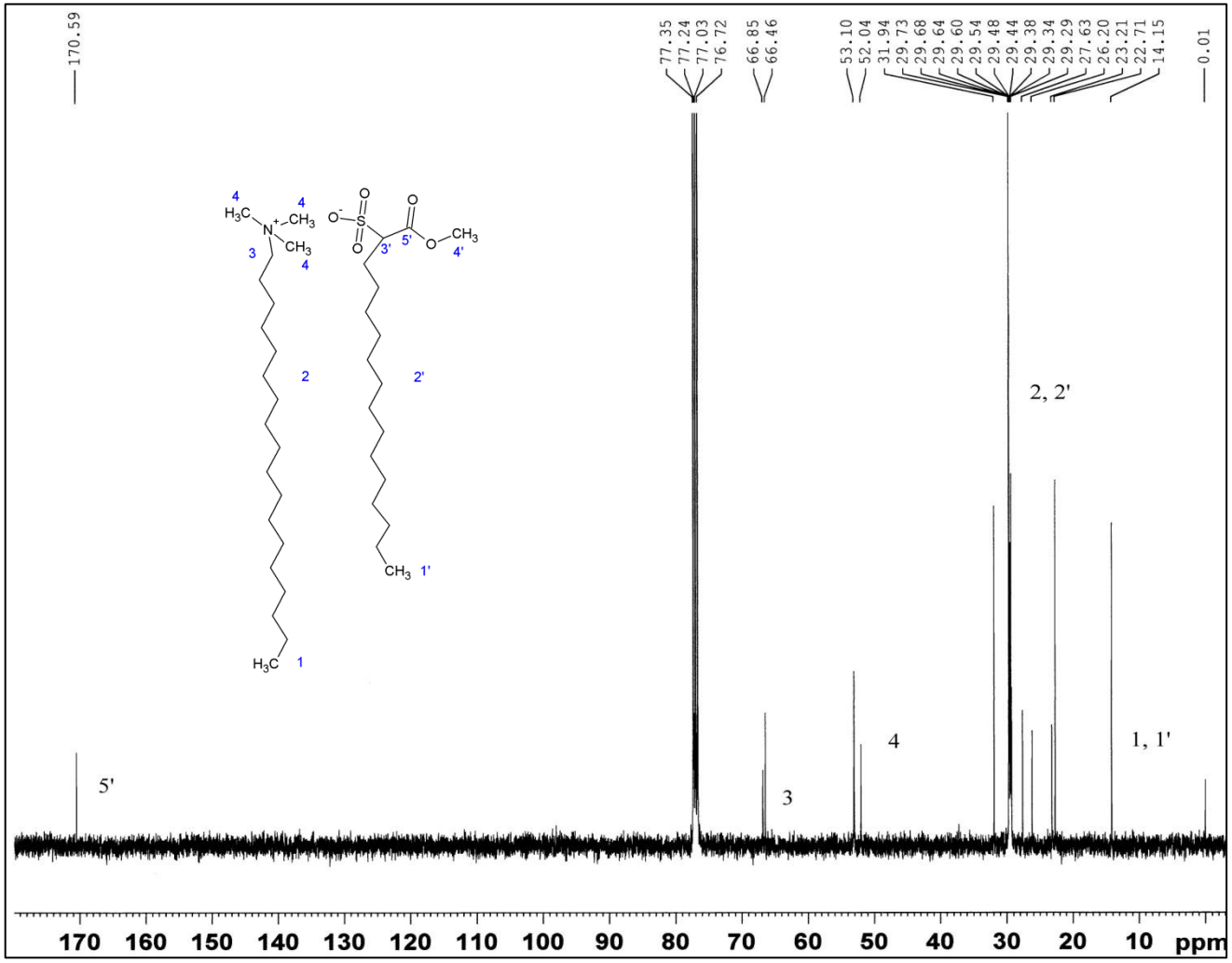
Appendix 25. ^{13}C NMR spectrum of $C_{14}MES.C_{12}TAB$ catanionic surfactant recorded at 125 MHz.



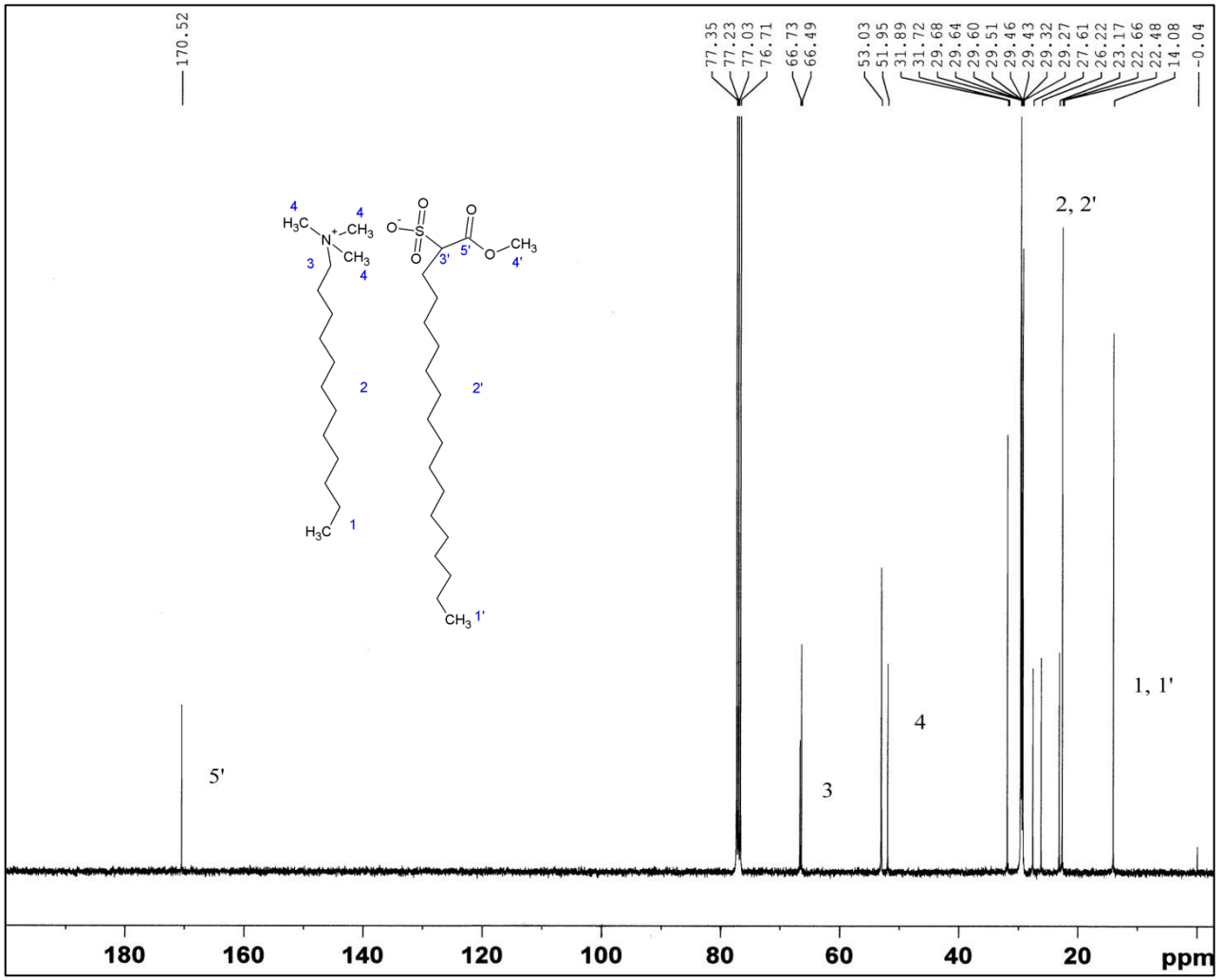
Appendix 26. ^{13}C NMR spectrum of $C_{14}MES.C_{14}TAB$ catanionic surfactant recorded at 125 MHz.



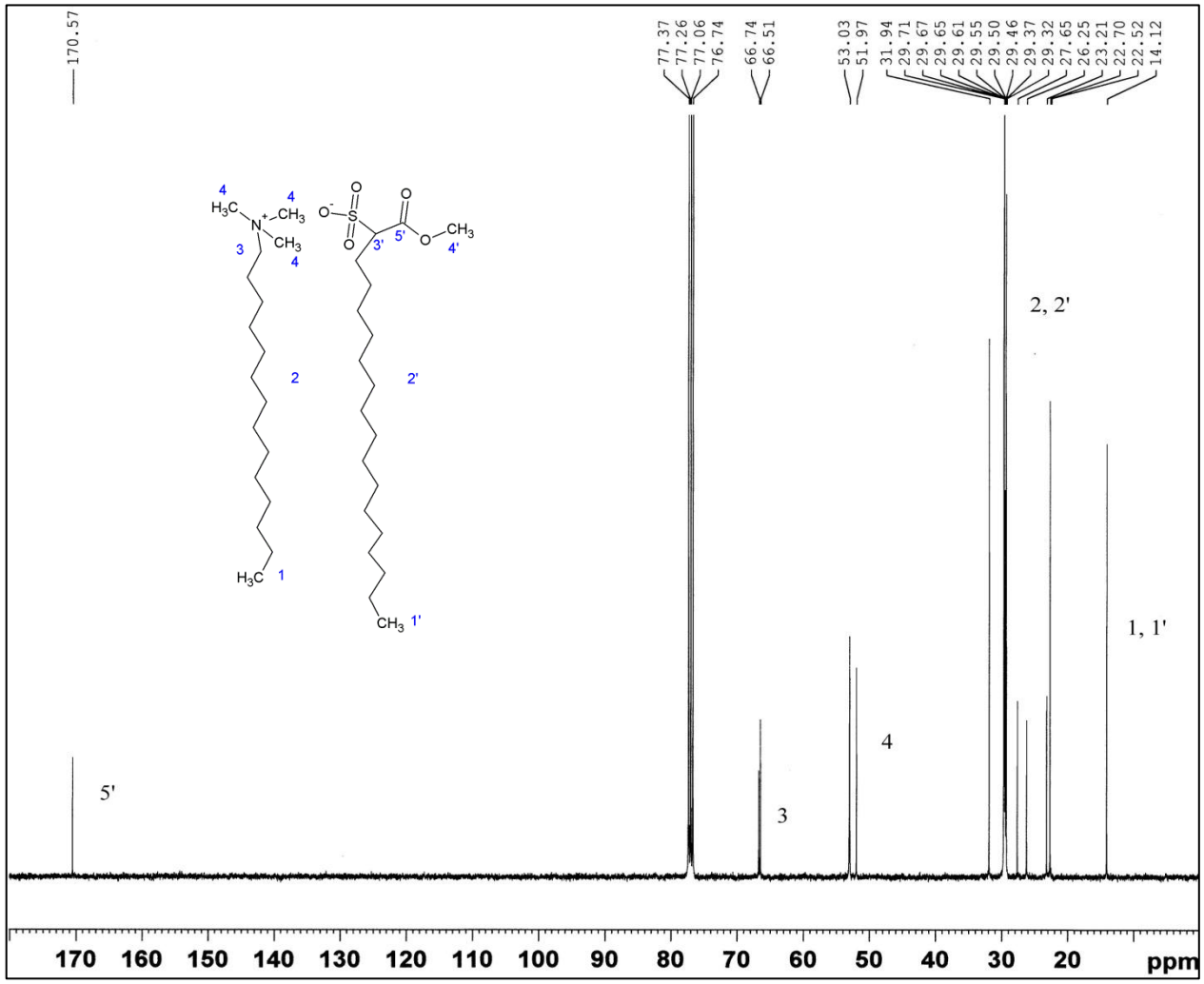
Appendix 27. ^{13}C NMR spectrum of $C_{14}MES.C_{16}TAB$ catanionic surfactant recorded at 125 MHz.



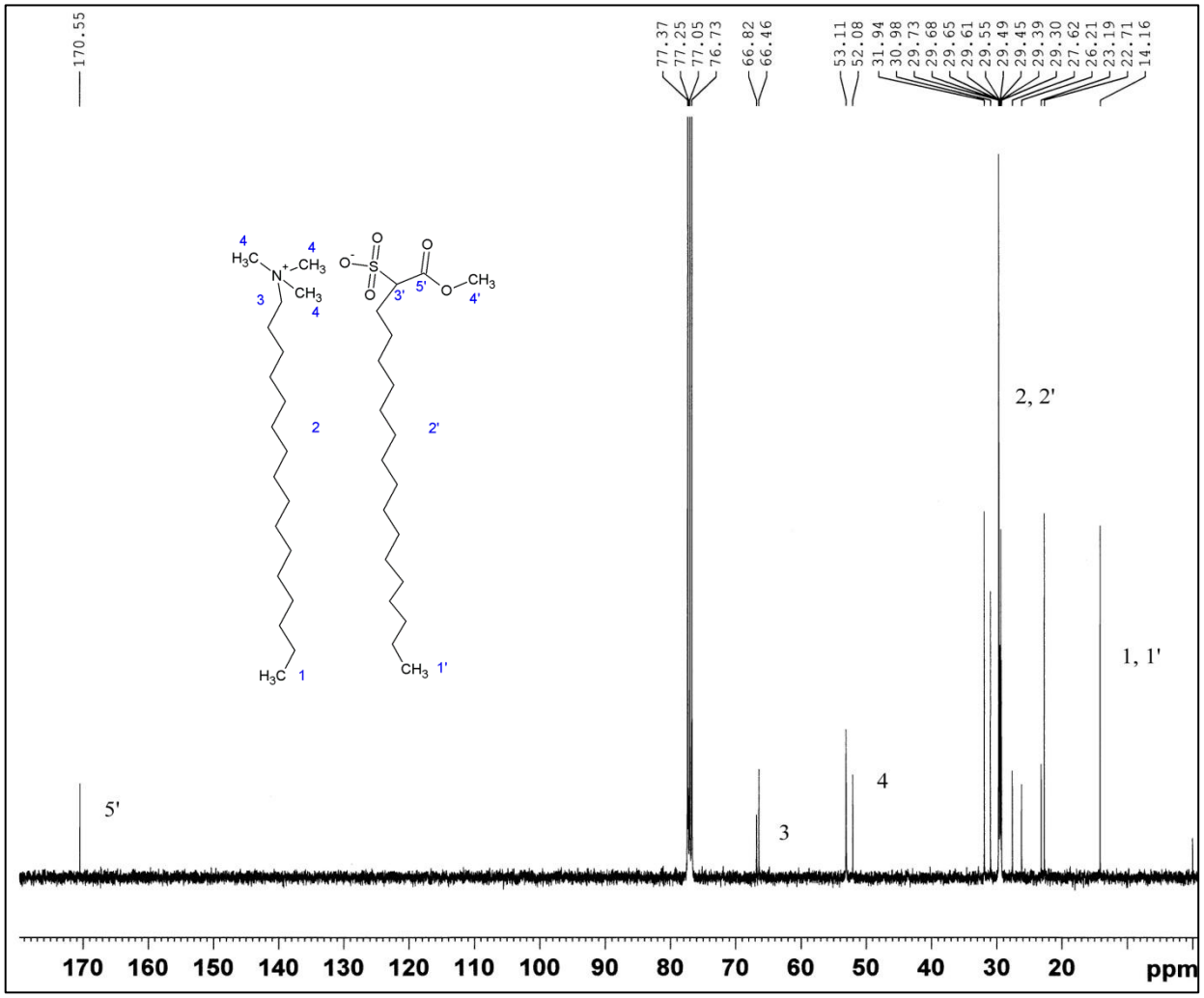
Appendix 28. ^{13}C NMR spectrum of $C_{14}MES.C_{18}TAB$ catanionic surfactant recorded at 125 MHz.



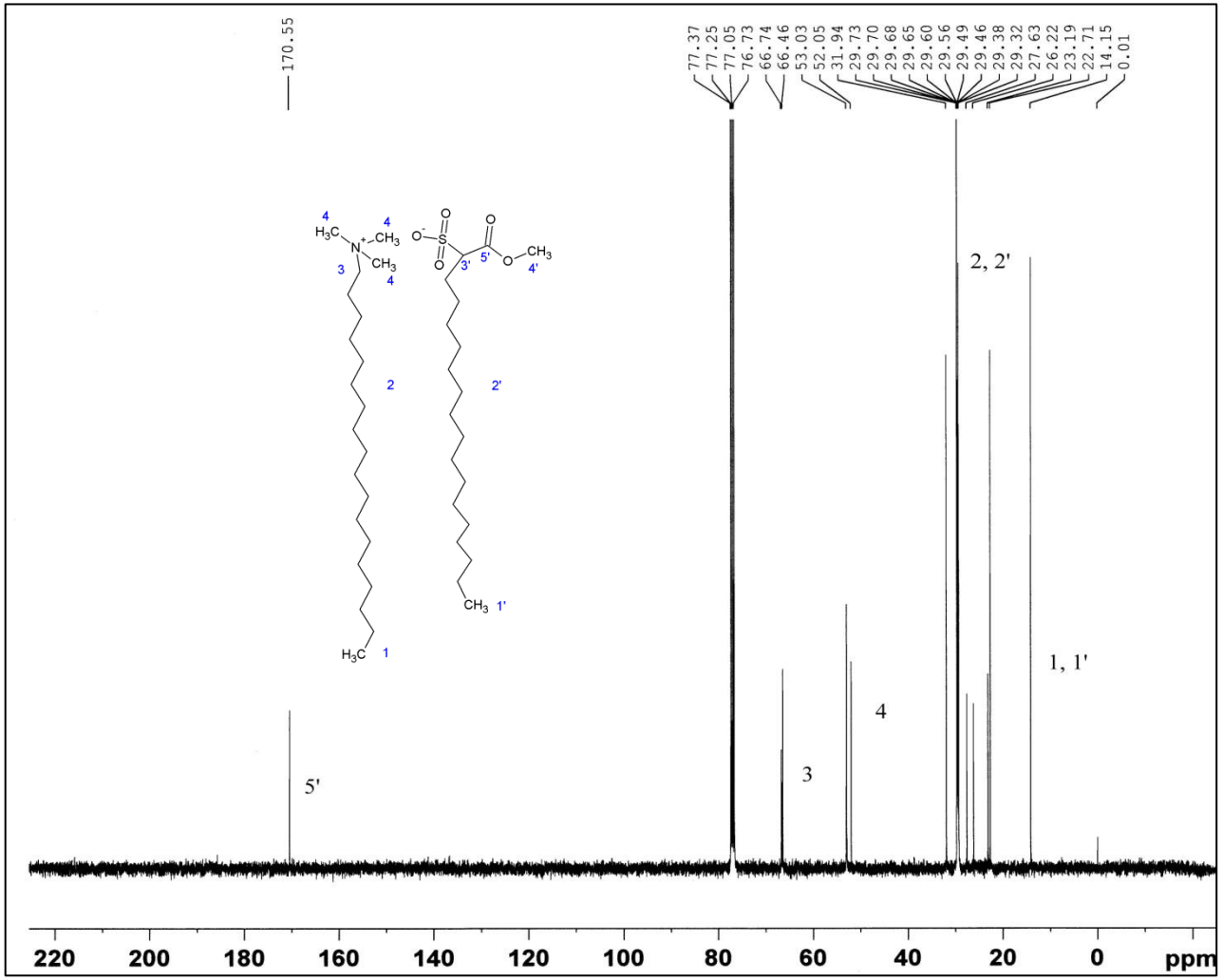
Appendix 29. ^{13}C NMR spectrum of $C_{16}MES.C_{12}TAB$ catanionic surfactant recorded at 125 MHz.



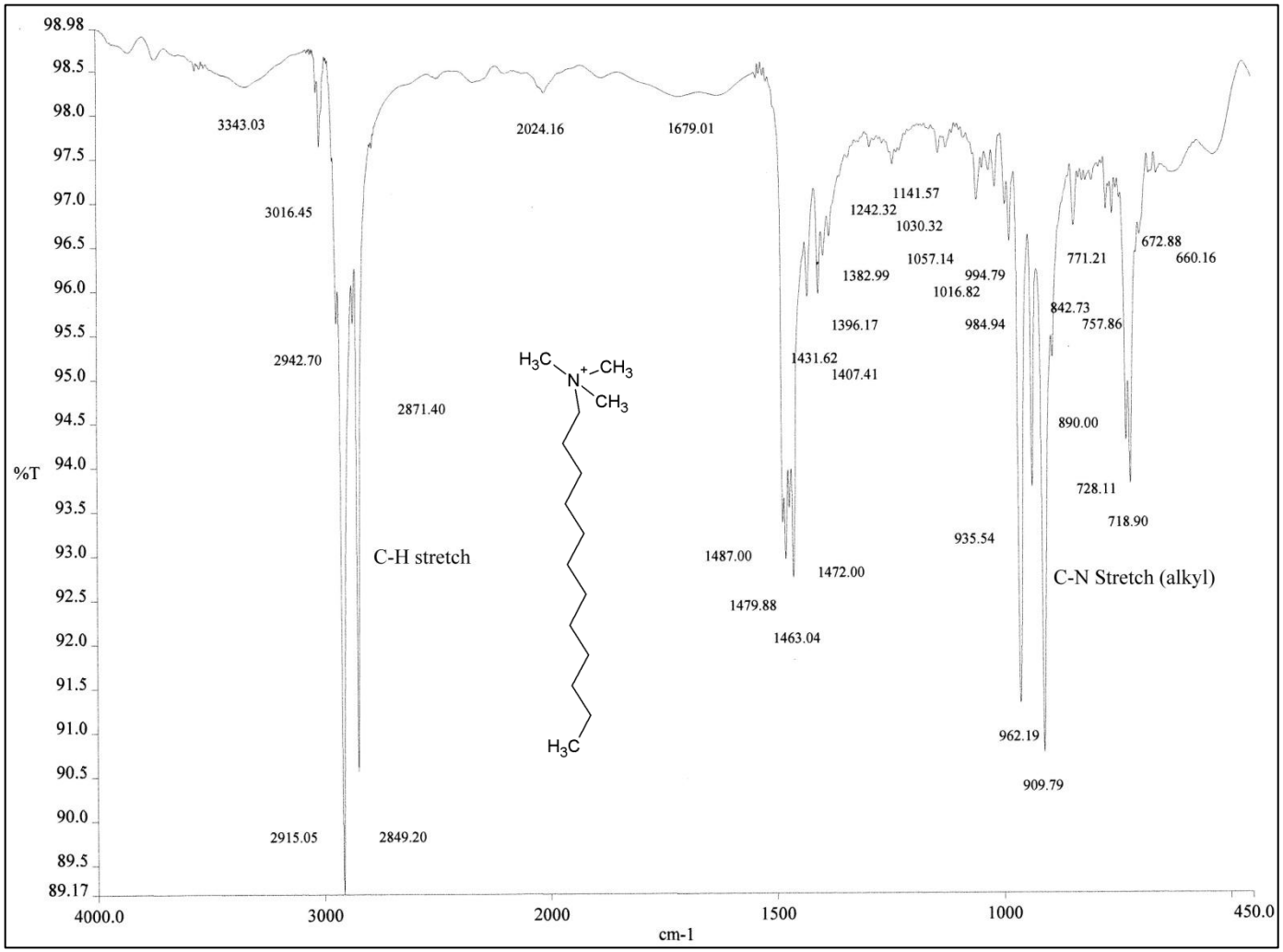
Appendix 30. ^{13}C NMR spectrum of $C_{16}MES.C_{14}TAB$ catanionic surfactant recorded at 125 MHz.



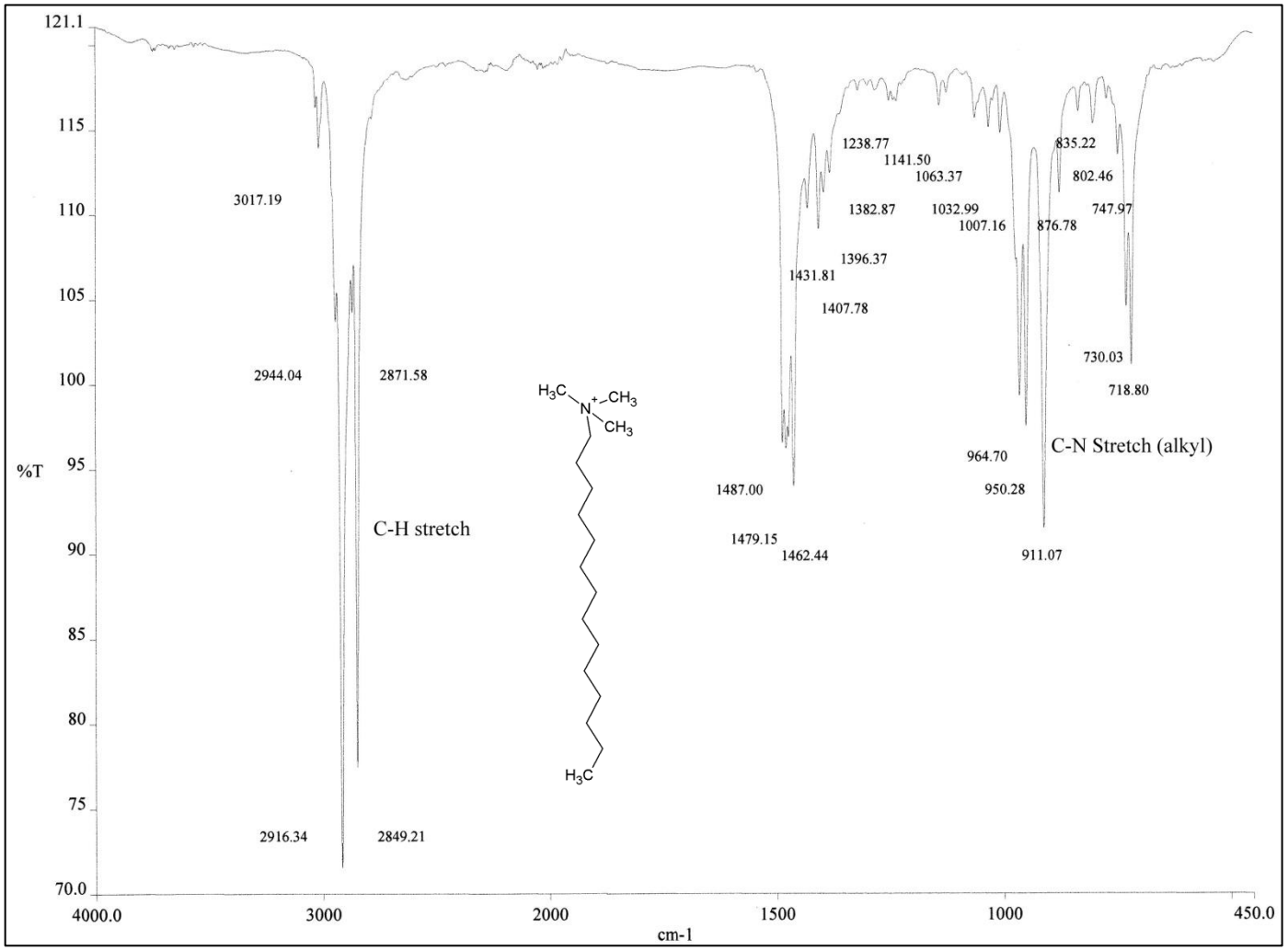
Appendix 31. ^{13}C NMR spectrum of $C_{16}MES.C_{16}TAB$ catanionic surfactant recorded at 125 MHz.



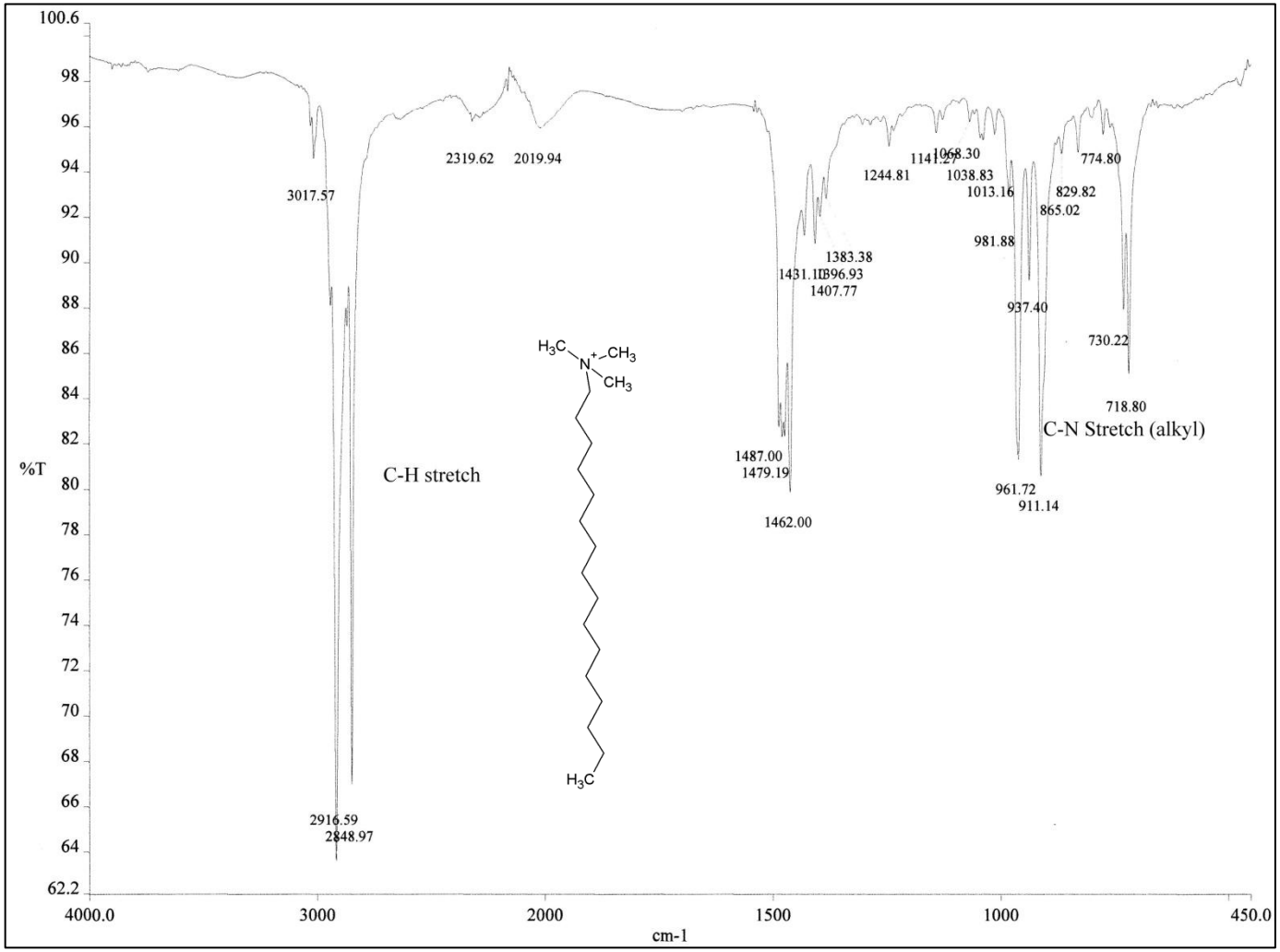
Appendix 32. ^{13}C NMR spectrum of $C_{16}MES.C_{18}TAB$ catanionic surfactant recorded at 125 MHz.



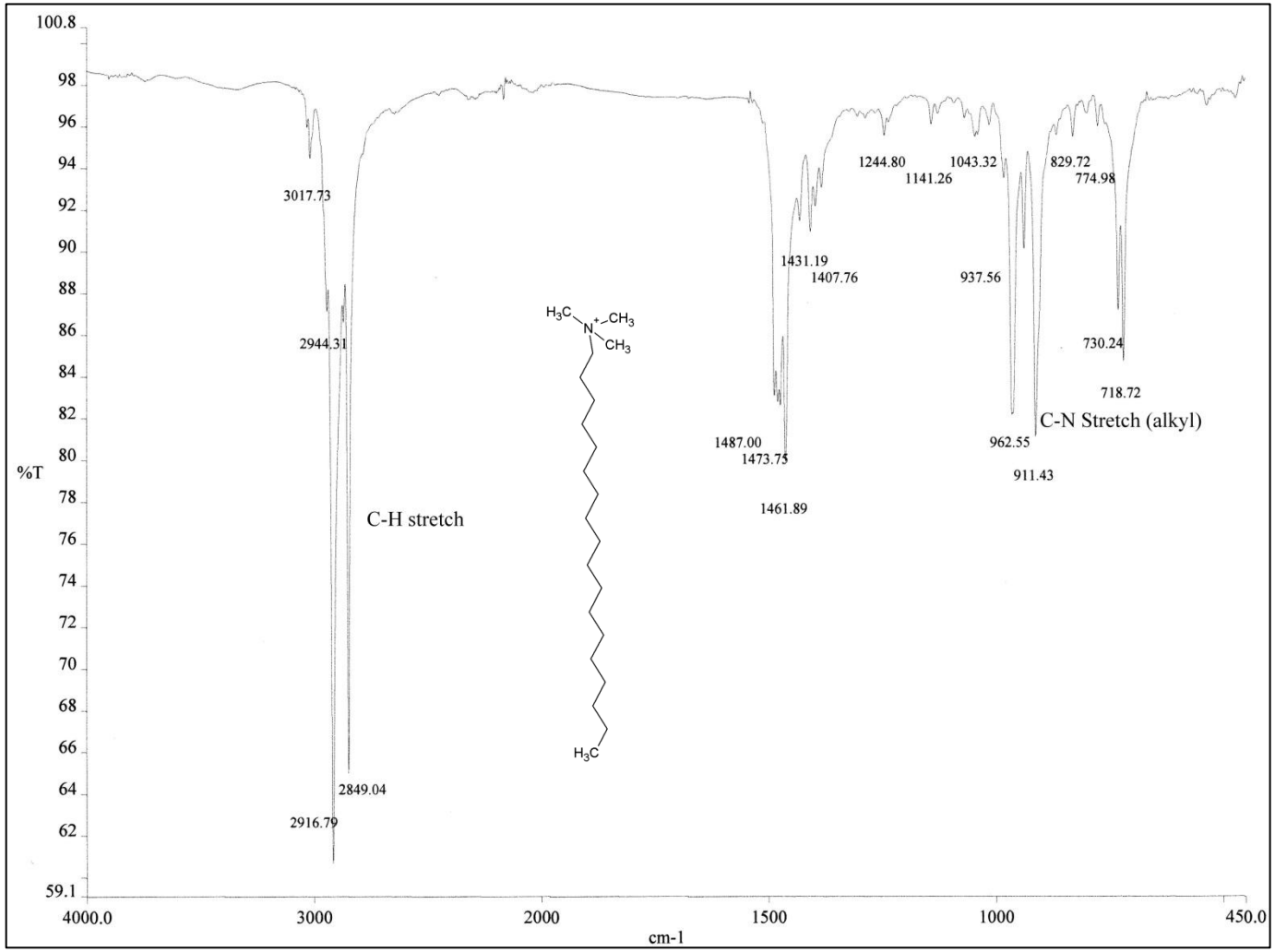
Appendix 33 FTIR spectrum of C_{12}TAB single surfactant recorded at 298K.



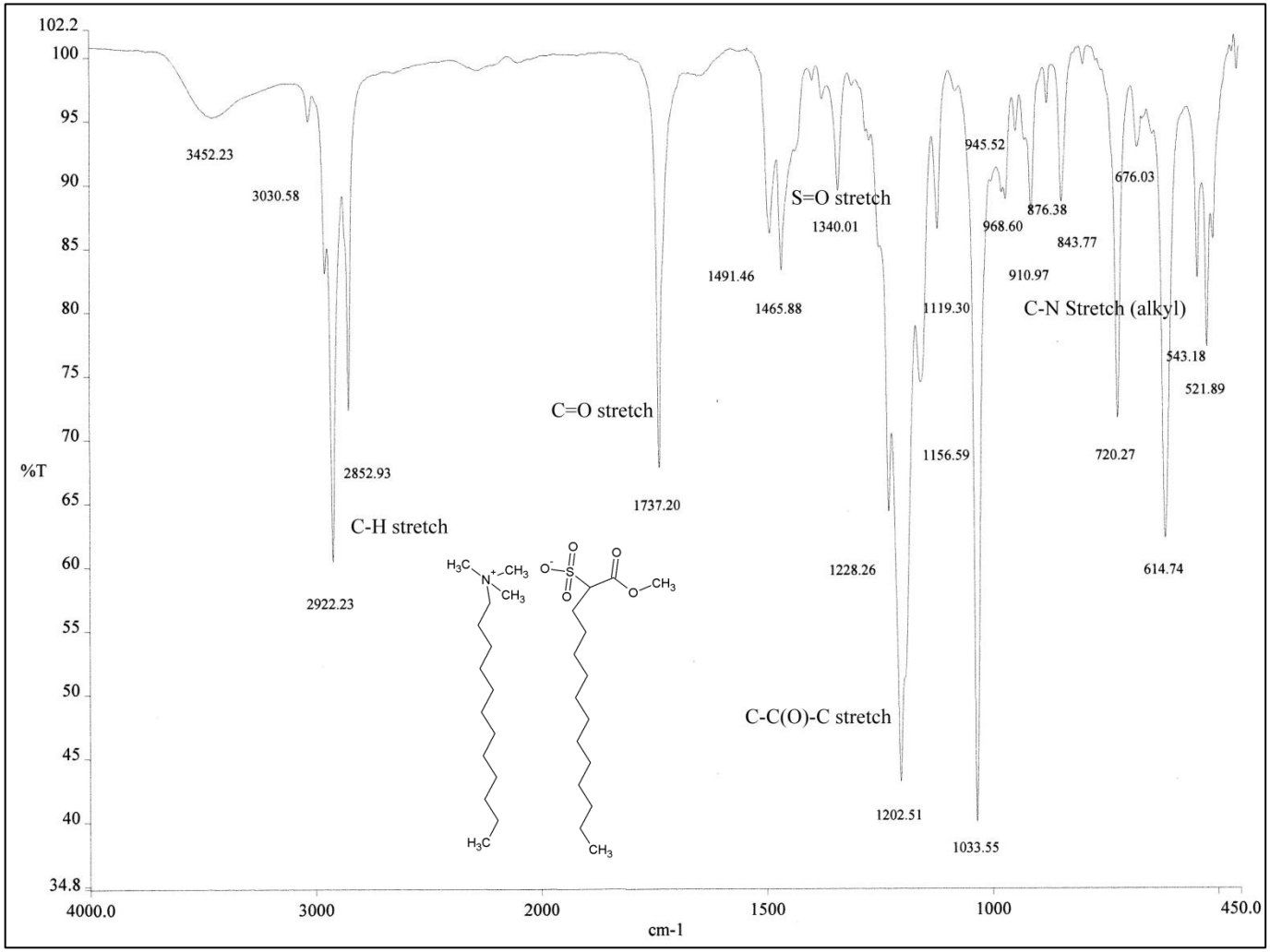
Appendix 34 FTIR spectrum of C_{14}TAB single surfactant recorded at 298K.



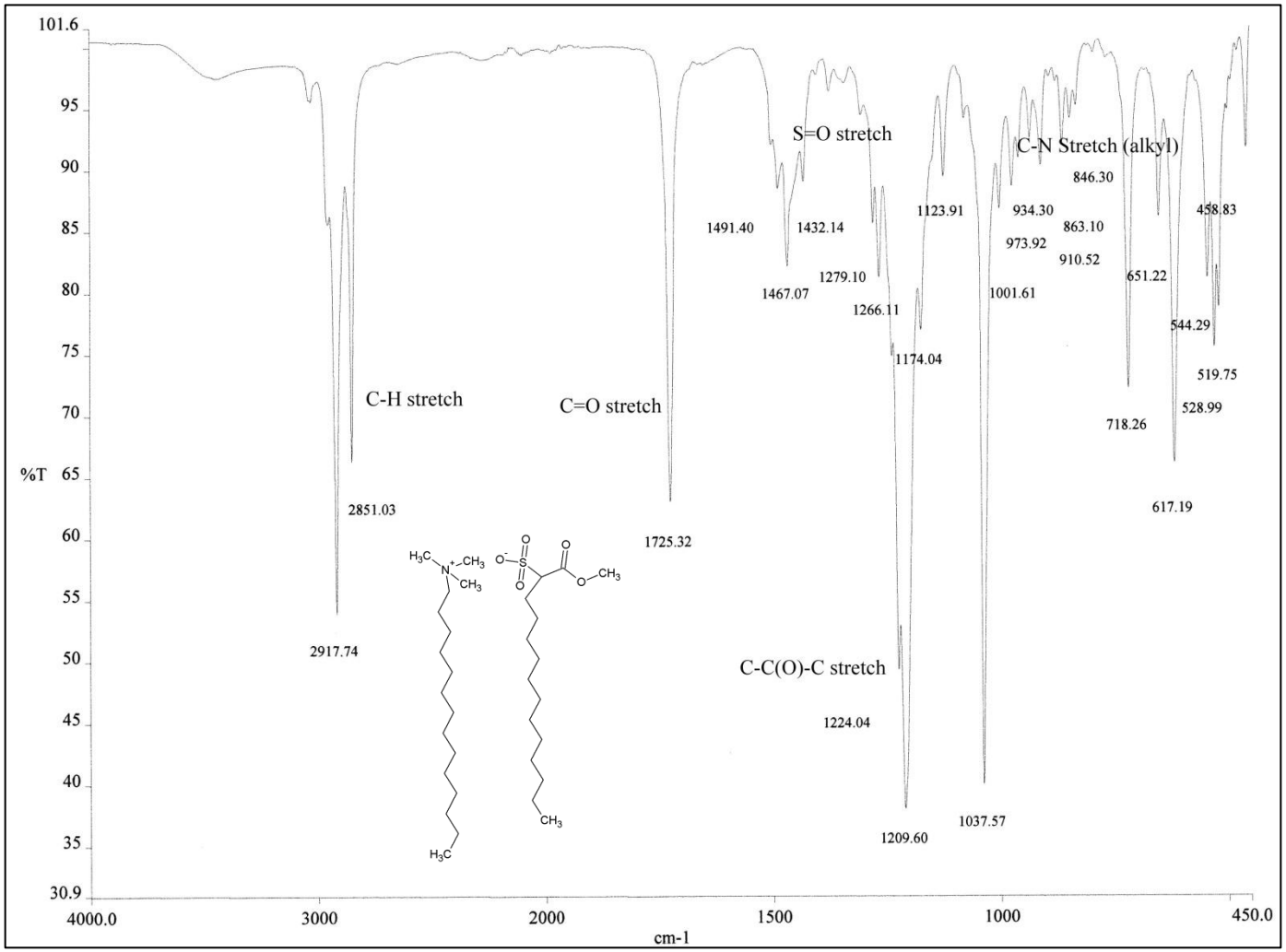
Appendix 35 FTIR spectrum of C_{16}TAB single surfactant recorded at 298K.



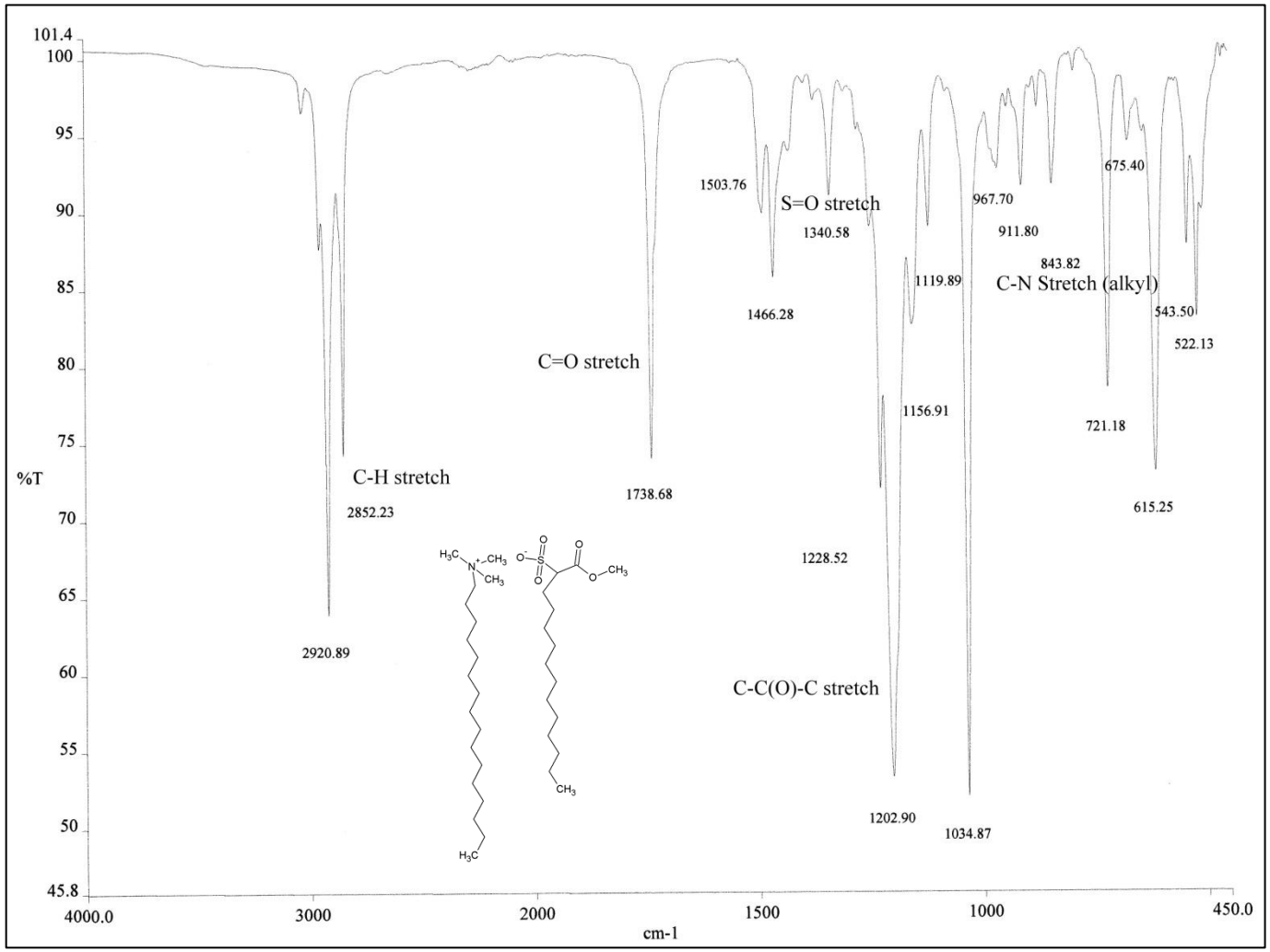
Appendix 36 FTIR spectrum of C_{18}TAB single surfactant recorded at 298K.



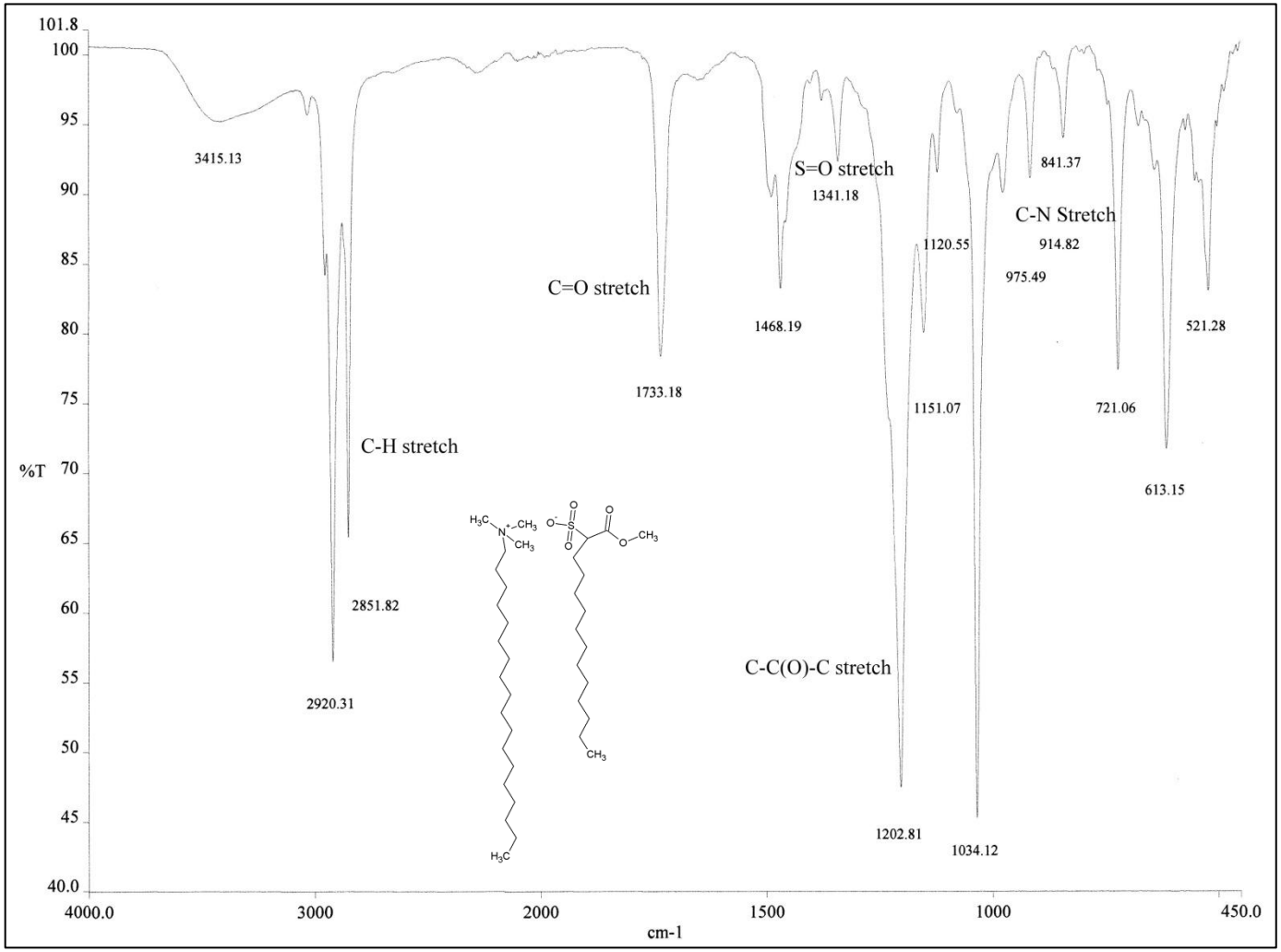
Appendix 37 FTIR spectrum of C_{12}MES.C_{12}TAB catanionic surfactant recorded at 298K.



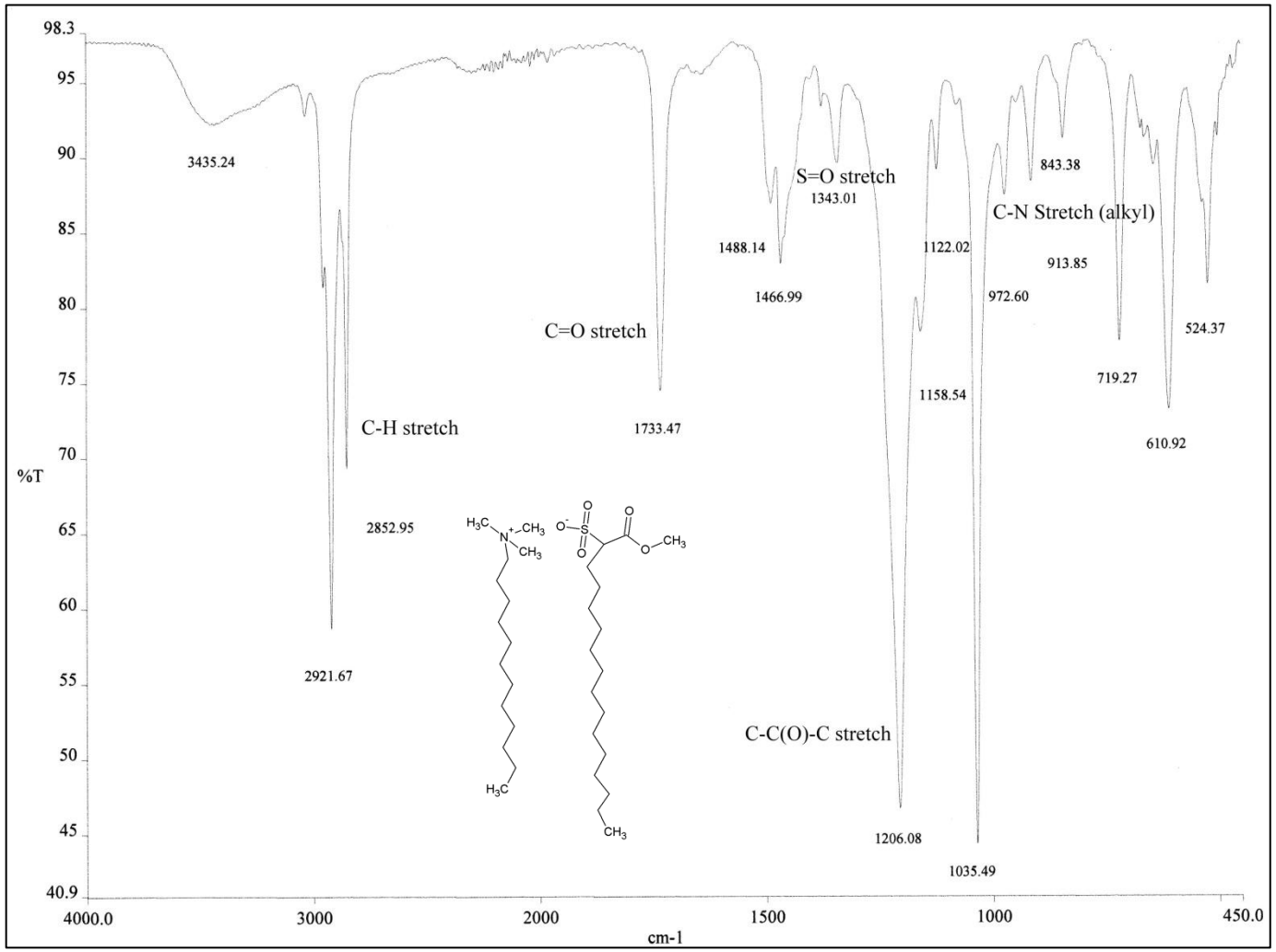
Appendix 38 FTIR spectrum of C_{12}MES.C_{14}TAB catanionic surfactant recorded at 298K.



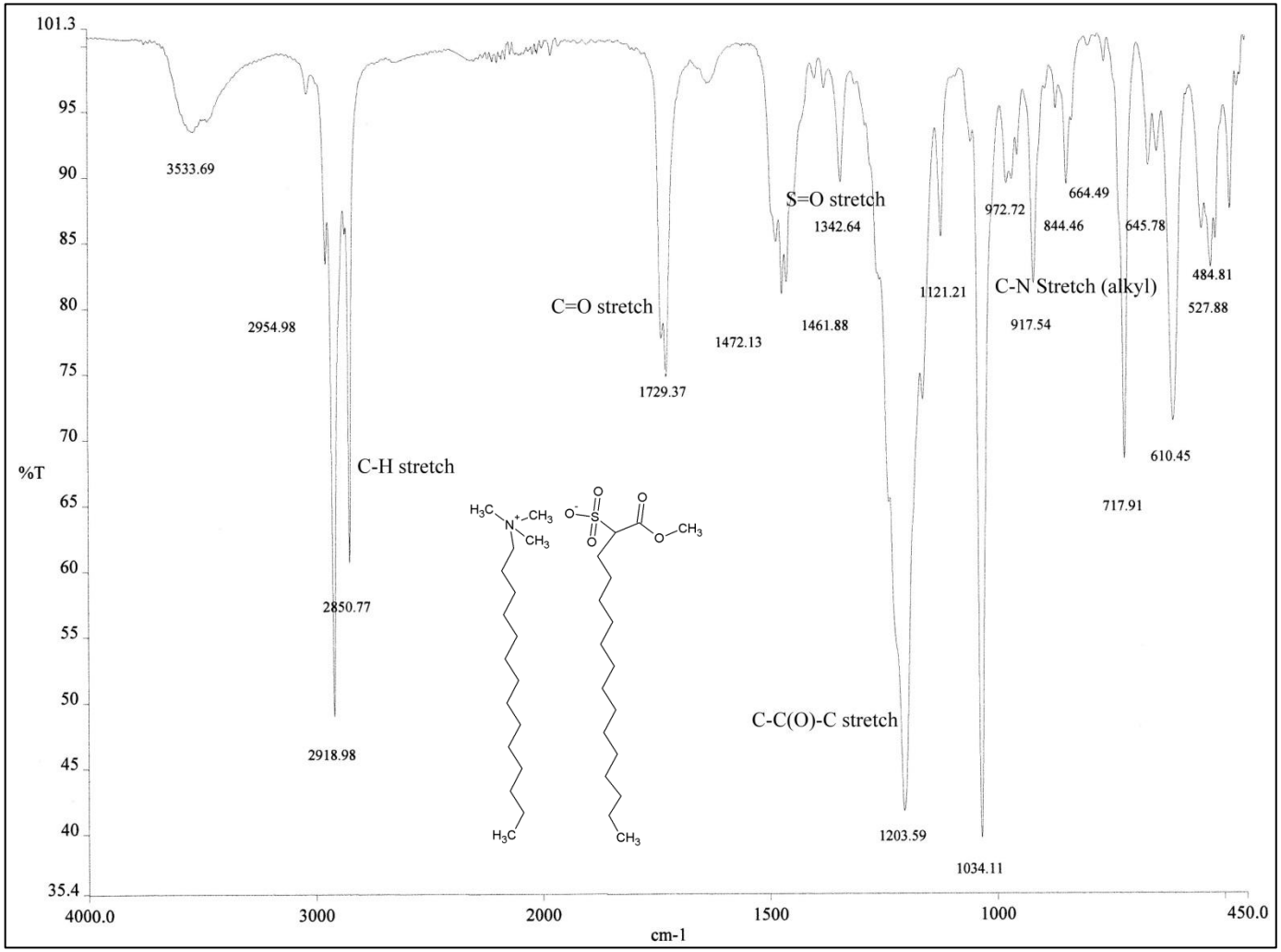
Appendix 39 FTIR spectrum of C_{12}MES.C_{16}TAB catanionic surfactant recorded at 298K.



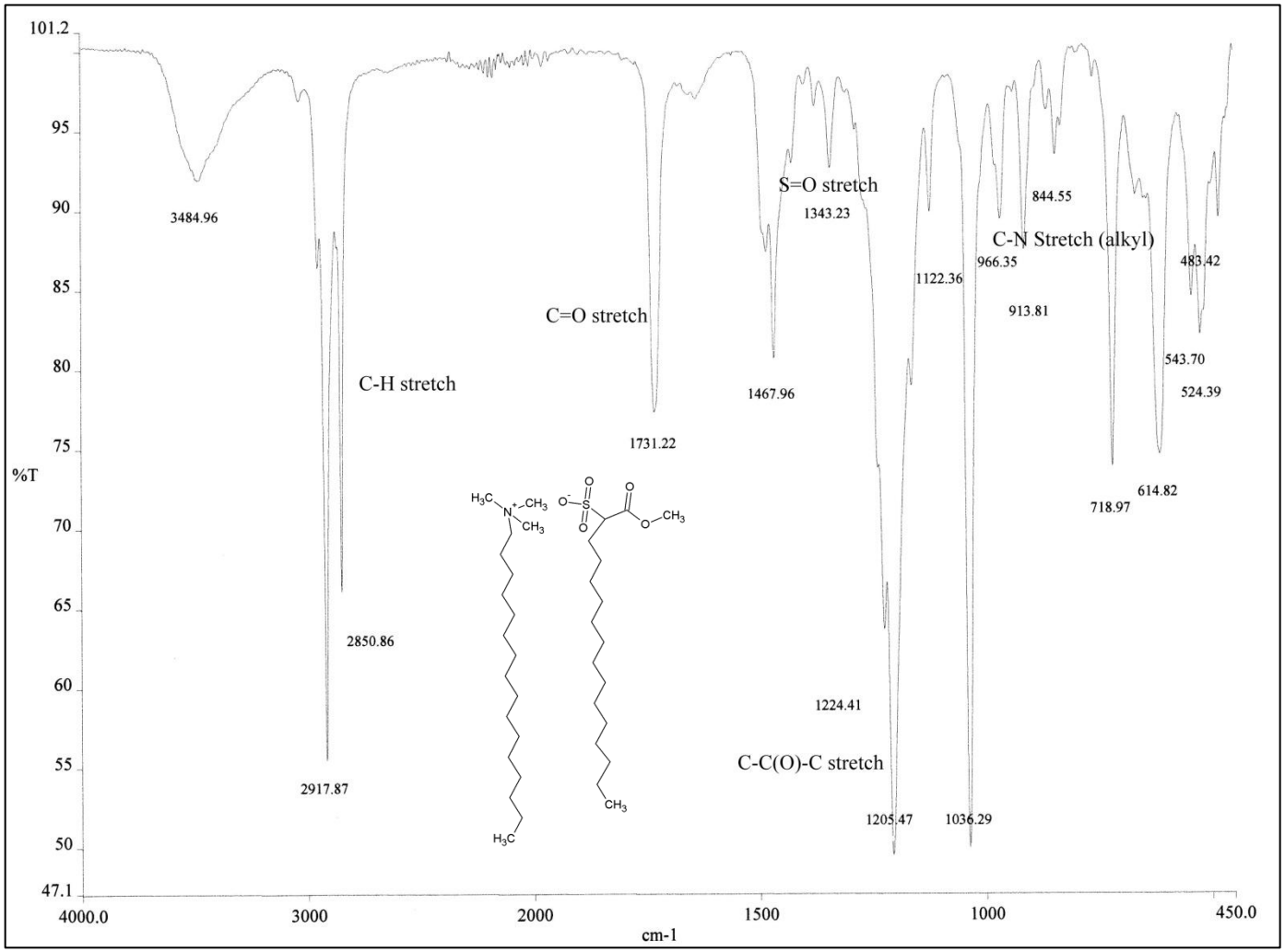
Appendix 40 FTIR spectrum of C_{12}MES.C_{18}TAB catanionic surfactant recorded at 298K.



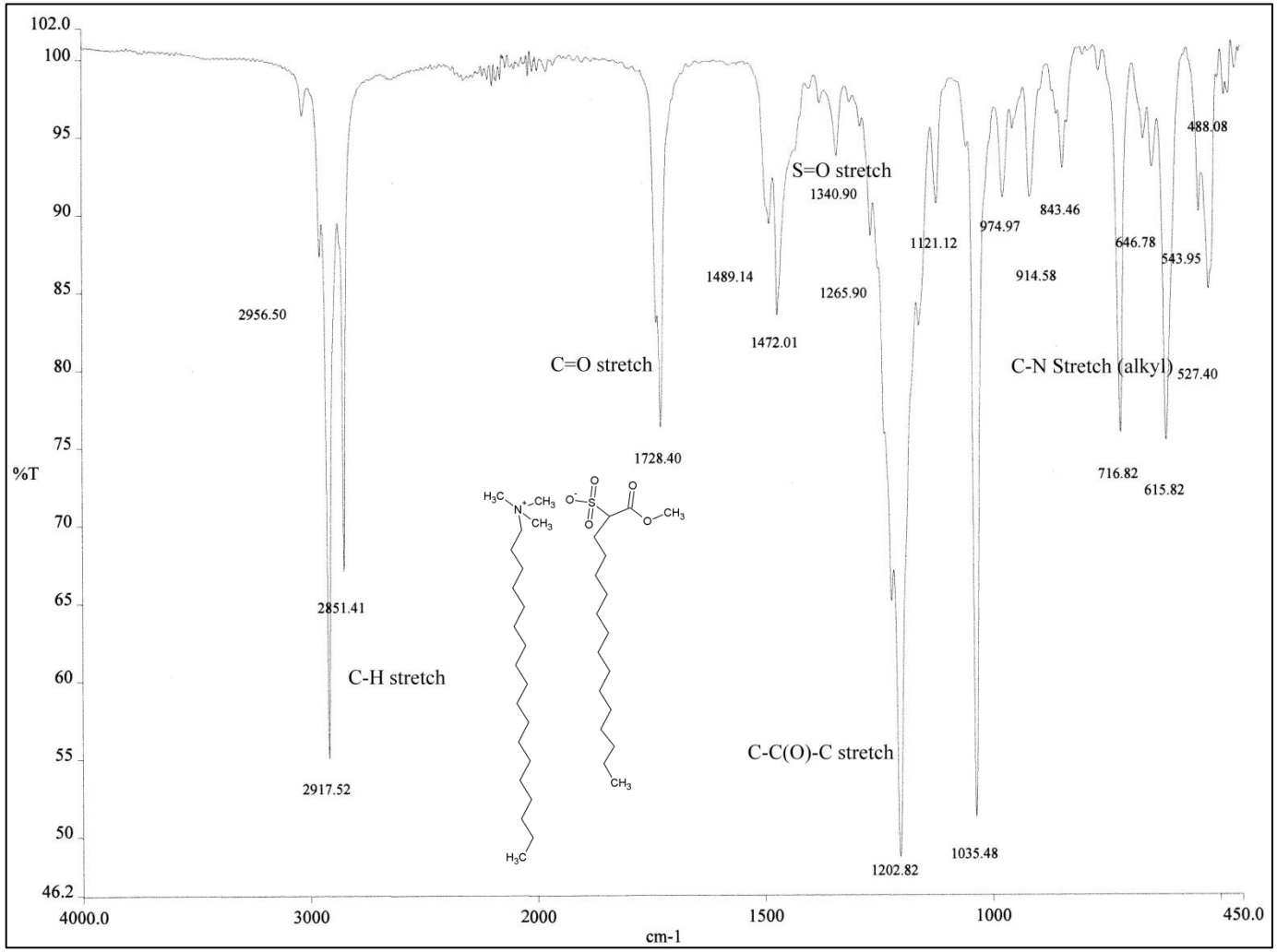
Appendix 41 FTIR spectrum of C_{14}MES.C_{12}TAB catanionic surfactant recorded at 298K.



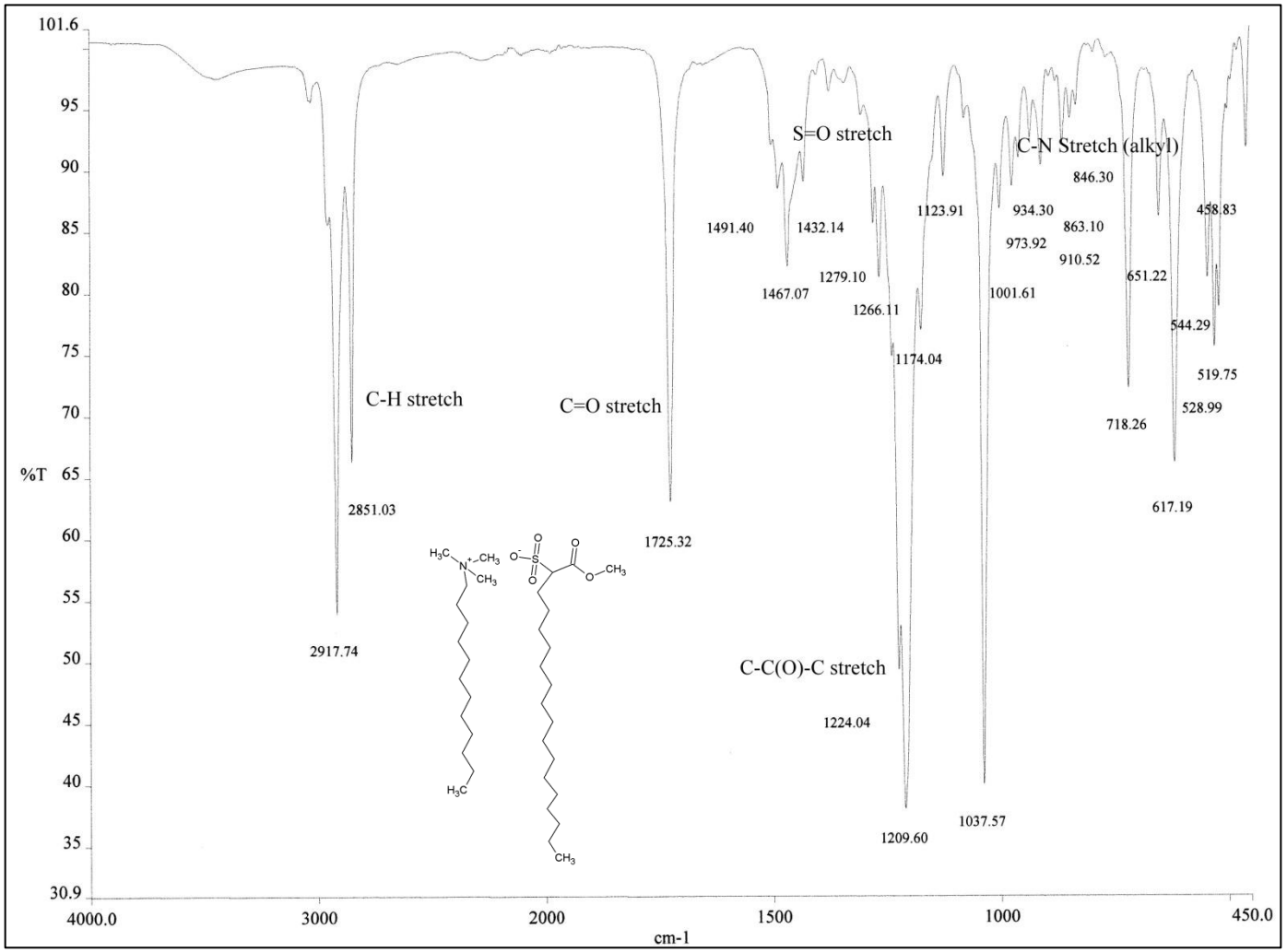
Appendix 42 FTIR spectrum of C_{14}MES.C_{14}TAB catanionic surfactant recorded at 298K.



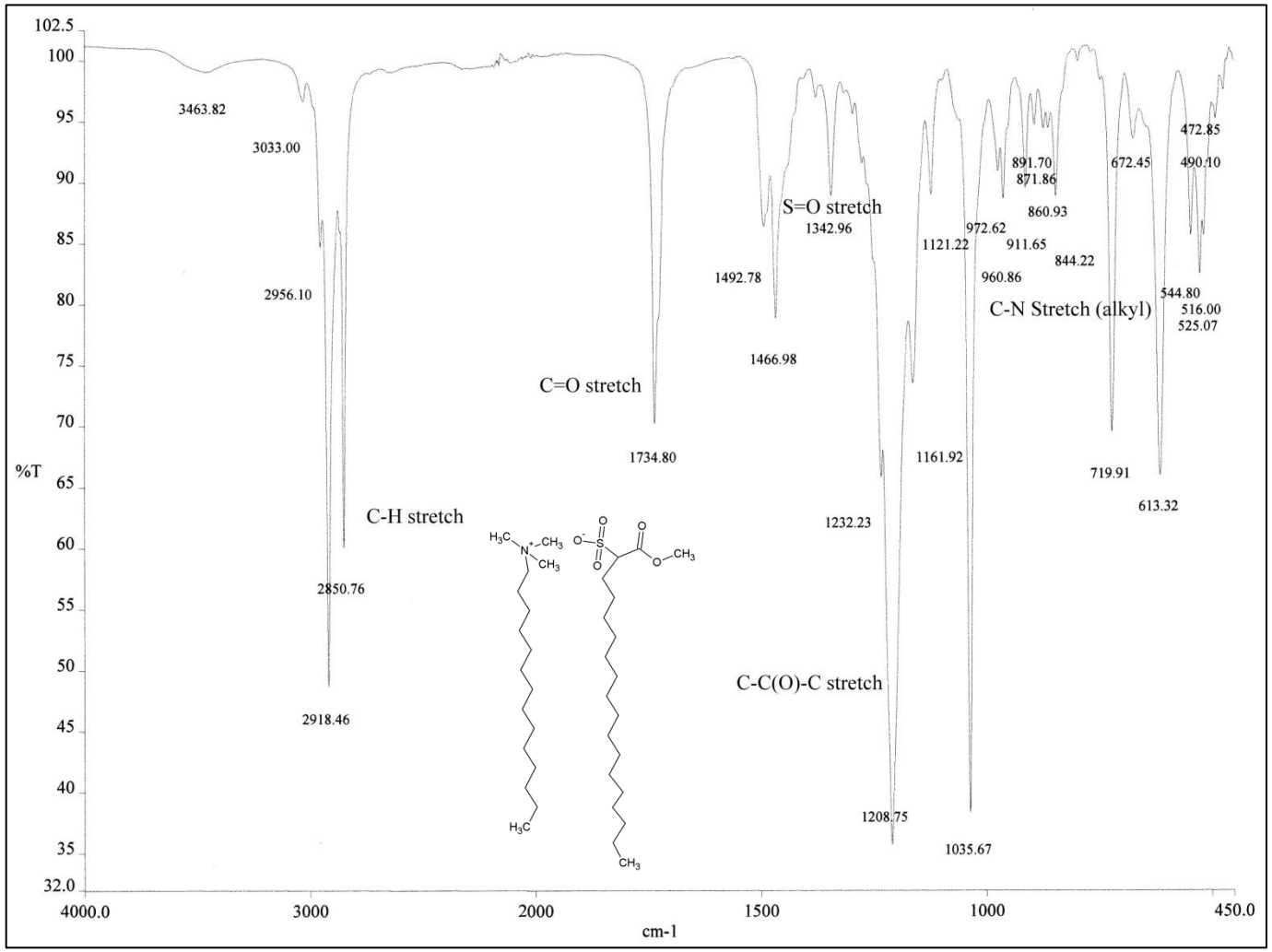
Appendix 43 FTIR spectrum of C_{14}MES.C_{16}TAB catanionic surfactant recorded at 298K.



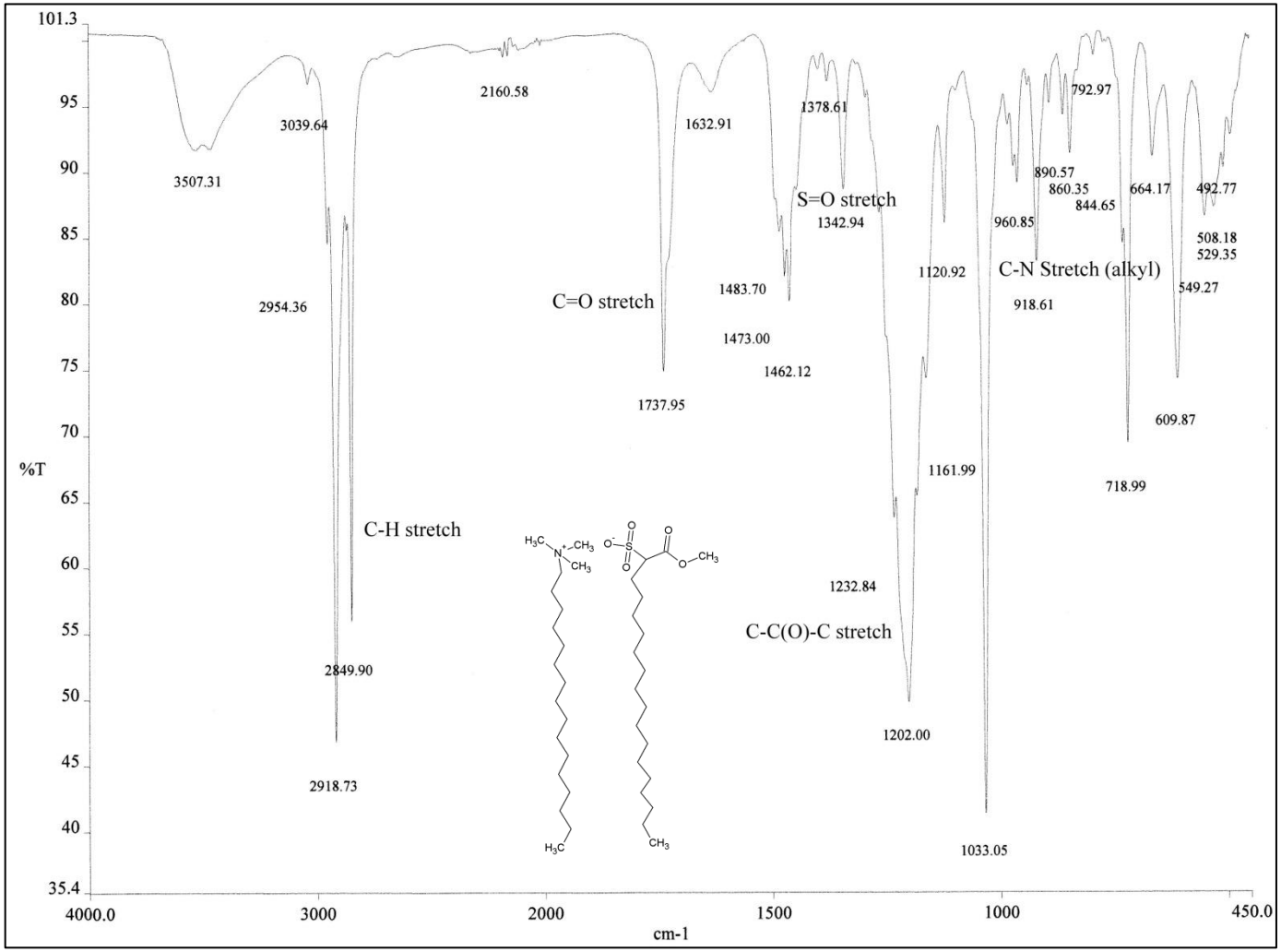
Appendix 44 FTIR spectrum of C_{14}MES.C_{18}TAB catanionic surfactant recorded at 298K.



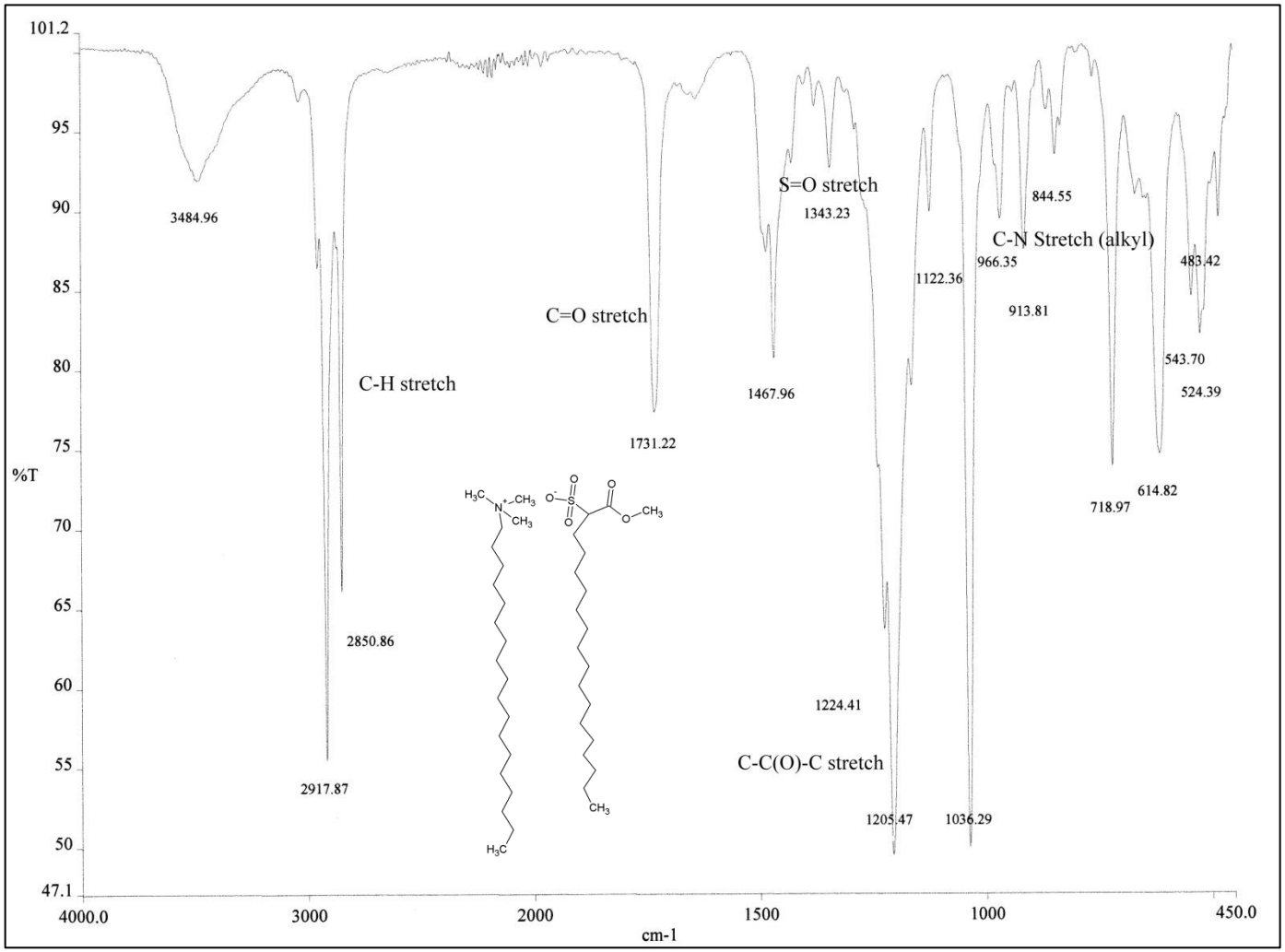
Appendix 45 FTIR spectrum of C_{16}MES.C_{12}TAB catanionic surfactant recorded at 298K.



Appendix 46 FTIR spectrum of C_{16}MES.C_{14}TAB catanionic surfactant recorded at 298K.



Appendix 47 FTIR spectrum of C_{16}MES.C_{16}TAB catanionic surfactant recorded at 298K.



Appendix 48 FTIR spectrum of C_{16}MES.C_{18}TAB catanionic surfactant recorded at 298K.